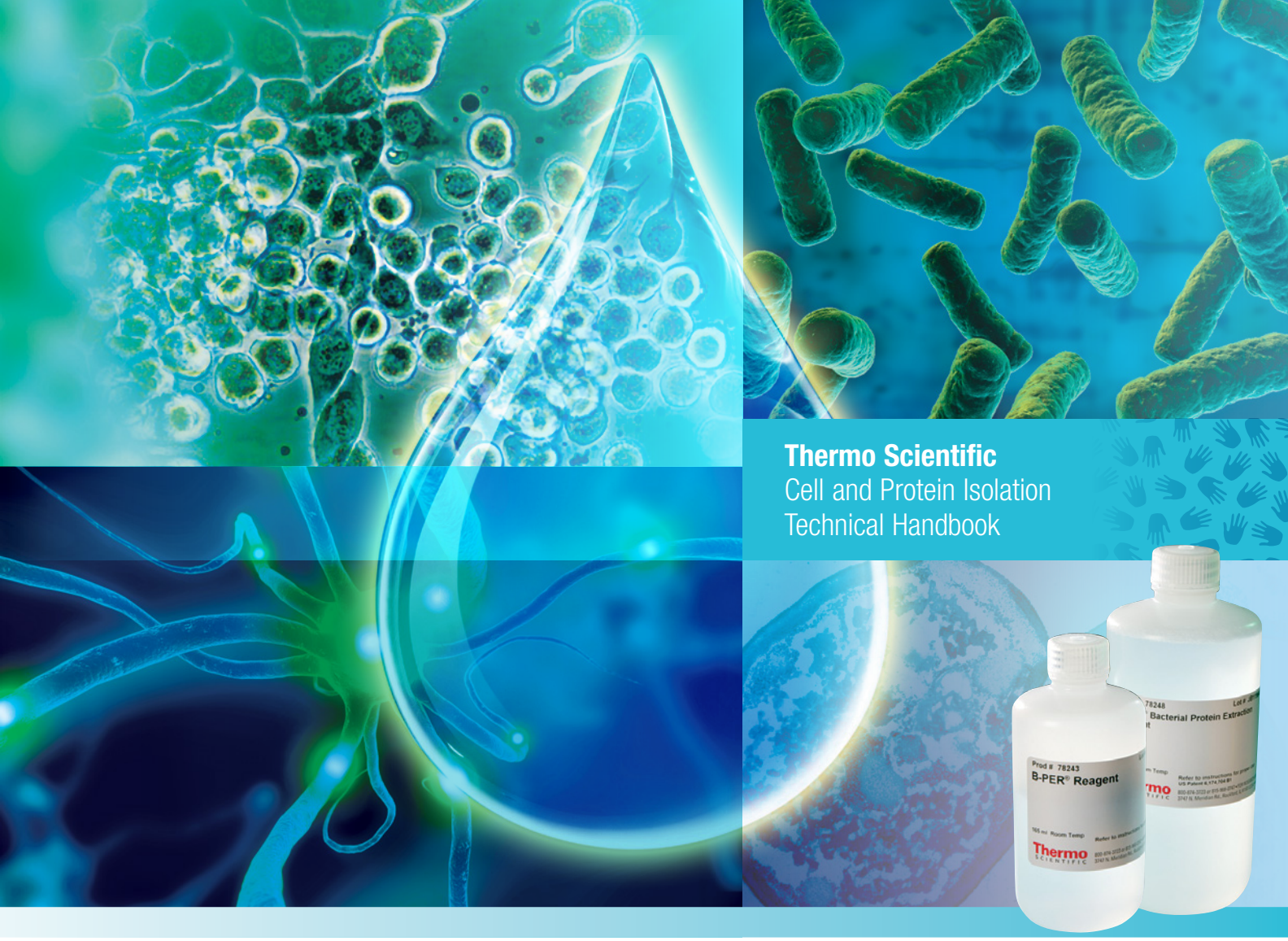

Tools and reagents for optimal protein extraction

• cell isolation • cell lysis • cell fractionation • protease and phosphatase inhibitors • detergents and detergent removal

Thermo SCIENTIFIC

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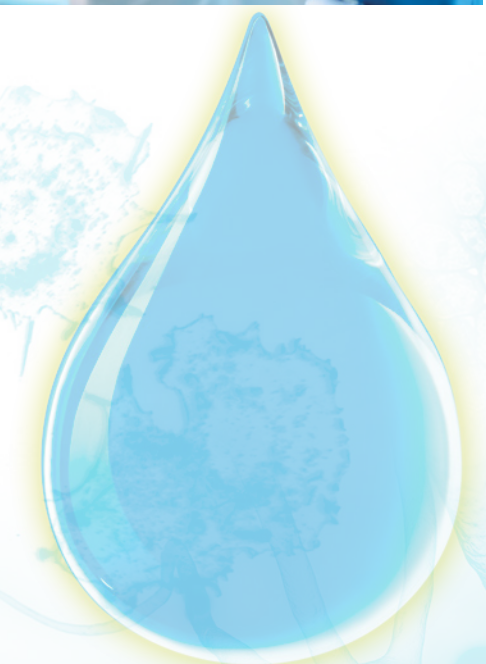
Overview of Protein Extraction from Cells and Tissues

The study of proteins in living organisms is an integral part of life science research. Proteins are the most diverse group of biologically important molecules and are essential for cellular structure and function. The first step in protein analysis is cellular extraction. Because proteins are so heterogeneous, there is no one method or reagent that is optimal for general protein isolation. In addition, protein extraction techniques vary depending on the source of the starting material, the location within the cell of the protein of interest and the downstream application. Many techniques have been developed to obtain the best protein yield and purity for different types of cells and tissues, taking into account where appropriate, the subcellular location of the protein and the compatibility of the protein extract with the next step in the experiment.



Key Considerations for Protein Extraction

Sample type

In life science research, proteins are typically extracted from cultured mammalian cells, mammalian tissues or primary cells. When extracting proteins from mammalian tissues, mechanical disruption is required to isolate the cells from their tissue matrix. For cultured mammalian and primary cells, which have only a plasma membrane separating the cell contents from the environment, reagents containing detergents and other components can easily disrupt the protein-lipid membrane bilayer, making total protein extraction relatively straightforward. Other organisms that are also commonly used in protein research, including bacteria (as a tool for protein expression), yeast (as a model for cell biology) and plants (for agricultural biotechnology) contain cell walls, which have traditionally required mechanical lysis. However, detergent-based solutions have been developed to efficiently lyse these cells without using physical disruption. Table 1 describes the most common methods for protein extraction, as well as the advantages and disadvantages of each method.

Protein location

Because certain proteins are localized in specific organelles, protein yield and enrichment are greatly improved if the protein is extracted directly from its subcellular compartment or organelle. Some types of mechanical lysis alone can disrupt all cellular compartments, making it difficult to achieve subcellular fractionation. However, by the careful optimization of physical disruption and detergent-buffer formulations, procedures have been developed that enable the separation of subcellular structures. For example, with the appropriate detergents, hydrophobic membrane proteins can be solubilized and separated from hydrophilic proteins. A combination of tools and steps enables the isolation of intact nuclei, mitochondria and other organelles for study or protein solubilization.

Protein stabilization during extraction

Cell lysis disrupts cell membranes and organelles resulting in unregulated proteolytic activity that can reduce protein yield and function. To prevent degradation of extracted proteins and obtain the best possible protein yield and activity following cell lysis, protease and phosphatase inhibitors can be added to the lysis reagents. Numerous compounds have been identified and used to inactivate or block the activities of proteases and phosphatases by reversibly or irreversibly binding to them.

Because some detergents used in protein extraction formulations may inactivate the function of enzymes of interest or affect their long-term stability, it may be important to remove the detergents following cell lysis. In addition, high concentrations of detergents or salts can interfere with common research methods such as protein assays, protein purification, immunoprecipitation, gel electrophoresis and mass spectrometry (MS). In some cases, interfering substances can be mitigated simply by dilution or dialysis.

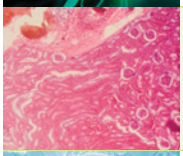
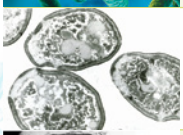
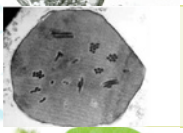
Table 1. Methods used for cell or tissue disruption.

Lysis Method	Apparatus	Method	Sample Type	Fractions Isolated	Advantages	Disadvantages
Reagent-based	None, except for tissues	Disruption of lipid membrane and/or cell wall	Mammalian, bacterial, yeast, insect, and plant cells and/or tissues	Total protein or subcellular fractions or organelles	Rapid, gentle, efficient, reproducible, high protein yield	Some components may need to be removed for downstream analysis
Mechanical	Waring blender	Shearing caused by rotating blades	Mammalian tissues or cells	Total protein	Inexpensive equipment	Reproducibility may vary, denaturing may occur, noisy
	Polytron™ mixer	Shearing occurs within long shaft containing rotating blades	Mammalian tissues or cells	Total protein	Inexpensive equipment	Reproducibility may vary, denaturing may occur
	Bead beater	Disruption caused by collision with agitated beads in liquid suspension	Mammalian tissues or cells, yeast or bacterial cells, plant tissue	Total protein	Low shearing, works with wide range of cell and tissue types, good for hard, difficult tissues	Denaturing may occur due to heat generated, noisy
	Dounce homogenizer	Shearing caused by a round glass pestle that is manually driven into a glass tube	Mammalian tissues or cells	Total protein, mitochondria	Inexpensive equipment, ideal for small volumes	Reproducibility may vary, denaturing may occur
	Potter-Elvehjem homogenizer	Shearing is caused by a PTFE-coated pestle that is manually or mechanically driven into a conical vessel	Mammalian tissues or cells	Total protein	Hand-held device, ideal for small volumes	Reproducibility may vary, denaturing may occur
	French press	Shearing is caused by high pressure when sample is forced through a small hole	Bacterial cells	Total protein	Rapid, efficient	Minimum 40mL samples, very expensive, noisy, lengthy set-up and clean-up
Sonication	Sonicator	Shearing is caused by high frequency sound waves	Mammalian tissues or cells, yeast or bacterial cells	Total protein	Directly compatible with downstream applications	Reproducibility may vary, denaturing may occur, noisy
Freeze/Thaw	Freezer or dry ice/ethanol in container	Disruption is caused by the formation of ice crystals in membranes	Bacterial and mammalian cells	Total protein	Inexpensive	Multiple cycles may be required, reproducibility may vary
Manual Grinding	Mortar and pestle	Disruption is caused by shearing and ice crystals in membranes	Plant tissue	Total protein	Inexpensive	Reproducibility may vary, laborious

Thermo Scientific™ Tools for Cell Isolation and Protein Extraction

Historically, physical lysis was the method of choice for protein extraction. However in recent years, detergent-based lysis methods have become standard. Our scientists have developed a broad portfolio of complete and ready-to-use reagents and kits for efficient cell isolation and protein extraction. The protein extraction reagents are optimized for specific cell and tissue types, the cellular location of the protein(s) of interest, and in most cases, do not require physical lysis. In addition, these products are compatible with the most commonly used downstream protein biology applications.

Table 2. Overview of sample types and Thermo Scientific Cell Isolation and Protein Extraction Reagents and Kits.

	Sample Type	Goal	Recommended Thermo Scientific Reagents or Kits	Product Information and Data
	Brain, Heart, Mouse Embryonic Fibroblast Tissue	Primary Cell Isolation	Pierce Primary Cell Isolation Kits for Neurons, Cardiomyocytes or MEFs	p. 8-12
	Primary or Cultured Mammalian Cells, or Tissues	Total Protein Extraction	M-PER Reagent T-PER Reagent N-PER Reagent Pierce RIPA Buffer Pierce IP Lysis Buffer	p. 13-18
	Cultured Mammalian Cells or Tissues	Subcellular Fractionation or Organelle Isolation	NE-PER Reagent Subcellular Fractionation Kits, Mitochondria Isolation Kits, Lysosome Enrichment Kit, and Cell Surface Protein Isolation Kits Syn-PER Reagent	p. 20-33
	Bacterial Cells	Total Protein Extraction	B-PER Reagent	p. 36
	Yeast Cells	Total Protein Extraction	Y-PER Reagent	p. 39
	Insect Cells (*Baculovirus*)	Total Protein Extraction	I-PER Reagent	p. 41
	Plant Tissue (Leaf, Stem, Roots, Flowers)	Total Protein Extraction	P-PER Reagent	p. 42

introduction

To better understand human diseases or pathways, life science researchers typically isolate proteins from cultured mammalian cells, tissues and/or primary cells. Mammalian cells are eukaryotic and include self-contained structures and organelles such as the nucleus, mitochondria and lysosomes.

Mammalian sample types

Cultured mammalian cells are the most commonly used source for protein research. These transformed ("immortalized") cell lines were developed in the 1950s and can be cultured indefinitely, due to altered growth properties. They have gained widespread use because they are easy to maintain, can be genetically modified, and can be scaled up quickly compared to tissues and primary cells. However, because they can contain genetic alterations, they can differ significantly from *in vivo* cell systems.

Tissues are composed of similar cells from the same origin that function together in a specialized activity. There are four types of tissues found in animals: epithelial, connective, nerve and muscle tissue. In addition to these common structures, cells can be even further differentiated with unique structures, such as the axons and dendrites in neural tissue.

The cells within tissues are tightly bound within an organ-specific matrix, requiring some form of physical disruption, such as mechanical shearing, liquid homogenization, sonication or snap freezing for protein extraction. Primary cells are isolated from neonatal or embryonic tissues using a combination of enzymes and mechanical dissociation. However, physical disruption is not required when extracting proteins from primary or cultured mammalian cells.

Detergent-based cell lysis is a milder and easier alternative to physical disruption of cell membranes. It is often used in conjunction with homogenization and mechanical grinding with a Polytron Mixer for tissue samples. Detergents break the lipid barrier surrounding cells by solubilizing proteins and disrupting lipid-protein interactions. In addition, detergent-buffer solutions, density gradient methods and/or other tools have been optimized to enable separation of subcellular structures or organelles.

Unfortunately, there is no standard protocol available for selecting a detergent to use for cell lysis and fractionation. The ideal detergent will depend on the intended application. In addition to the choice of detergent, other important considerations for optimal cell lysis include the buffer, pH, salt concentration and temperature. Consideration should be given to the compatibility of the chosen detergent with downstream applications.

Thermo Scientific Protein Isolation Products for Mammalian Tissues and Cells

Thermo Scientific total cell lysis, fractionation and organelle isolation reagents eliminate the need for hit-or-miss homemade recipes for protein isolation. All reagents and kits use gentle, non-denaturing detergents to prepare cell lysates and fractions that are compatible with typical downstream applications, such as protein assays, immunoprecipitation, electrophoresis and immunoassays. In addition, total cell extracts can be generated in less than 10 minutes, and up to five distinct subcellular fractions can be obtained in as few as 2-3 hours. Although most of the reagents and kits can be used for a variety of tissue and cell types, there are specialized reagents to extract proteins from neuronal tissues and cells. Protease and/or phosphatase inhibitor cocktails are always recommended when lysing cells to protect protein structure and phosphorylation states.

Primary cell isolation

The isolation of primary cells from tissues can be challenging. The number of times a primary cell culture can be passaged is minimal and requires expert manipulation and specialized media, serum, growth factors and culture conditions. Researchers must select and optimize many variables and conditions to grow these fragile cells. To assist with standardization of primary cell isolation, we offer complete, ready-to-use kits for the gentle and efficient isolation of primary neurons, cardiomyocytes and mouse embryonic fibroblasts (MEFs). These kits produce primary cells with higher yield and viability than most home-brew methods.

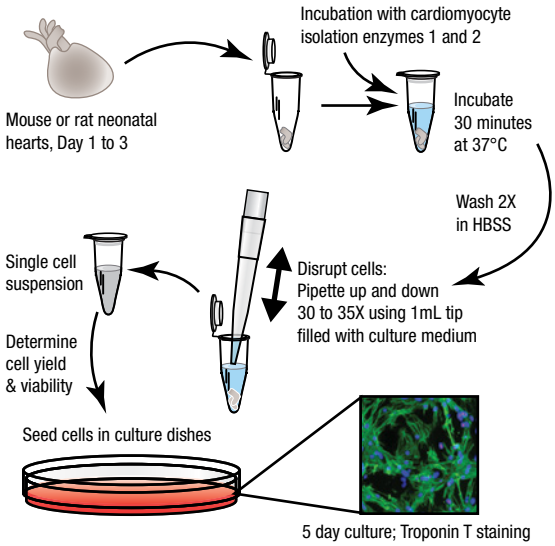


Figure 1. Schematic diagram of the Thermo Scientific Pierce Primary Cardiomyocyte Isolation Kit procedure.

Table 1. Overview of mammalian sample types and recommended Thermo Scientific Cell Isolation and Protein Extraction Reagents and Kits.

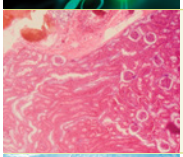
	Sample Type	Goal	Recommended Reagents or Kits	Product Information and Data
	Brain, Heart, Mouse Embryo Fibroblast Tissue	Primary Cell Isolation	Pierce Primary Cell Isolation Kits for Neurons, Cardiomyocytes or MEFs	p. 8-12
	Primary or Cultured Mammalian Cells, or Tissues	Total Protein Extraction	M-PER Reagent T-PER Reagent N-PER Reagent Pierce RIPA Buffer Pierce IP Lysis Buffer	p. 13-18
	Cultured Mammalian Cells or Tissues	Subcellular Fractionation or Organelle Isolation	NE-PER Reagent Subcellular Fractionation Kits, Mitochondria Isolation Kits, Lysosome Enrichment Kit, and Cell Surface Protein Isolation Kits Syn-PER Reagent	p. 20-33

Table 2. Product compatibility chart for protein extraction from mammalian tissues and cells.

				PROTEIN ASSAY COMPATIBILITY			
Kits	**Product #**	**Validated Tissues and Cell Lines**	**Component or Fraction**	**BCA**	**Commassie Plus**	**660nm**	**Detergent-compatible Bradford**
T-PER Reagent	**78510**	Brain, Heart, Liver, Kidney, Lung and Spleen Tissue	-	Yes	Yes	Yes	Yes
M-PER Reagent	**78501 78503 78505**	Brain and Kidney Tissue, Primary Cardiomyocytes and Mouse Embryonic Fibroblasts, HeLa, NIH 3T3, A431, A459, HEK293, Jurkat, FM2, HEPG2, HCT116, U20S, LNCaP, C2C12 and NS1 cells	-	Yes	Yes	Yes	Yes
RIPA Buffer	**89900 89901**	HeLa and A431 cells	-	Yes		No*	Yes, dilute 1:3
IP Lysis Buffer	**87787 87788**	Brain and Liver Tissue and HeLa, NIH 3T3, A431, A549, C6, HEK293, Jurkat, HepG2, HCT116, U20S, LNCaP, c2C12 and NS1 cells	-	Yes		Yes	Yes
N-PER Reagent	**87792**	Brain Tissue and Primary Neurons	-	Yes		Yes	Yes
Syn-PER Reagent	**87793**	Brain Tissue and Primary Neurons		Yes			
NE-PER Reagent	**78833 78835**	Heart, Liver, Kidney and Lung Tissue and HeLa, NIH 3T3, A549, C6, Jurkat and Cos7 cells	CER	Yes, dilute 1:4	Yes, dilute 1:4	Yes	Yes
			NER	Yes	Yes	Yes	Yes
Mem-PER Plus Reagent	**89843**	Brain, Heart, Liver and Kidney Tissue and HeLa, A431, A549, HEK293, Jurkat, FM2, HEPG2 and HCT116 cells	PB	Yes		Yes	Yes
			SB	Yes		Yes	Yes
Cell Surface Protein Isolation Kit	**89881**	HeLa, NIH 3T3, A431 and C6 cells		No	No	No*	No
Subcellular Fractionation Kit for Cultured Mammalian Cells or Tissues	**78840**	HeLa, NIH 3T3, A549 and HEK293 cells	CEB	Yes		Yes	Yes
			MEB	Yes		Yes	
			NEB	Yes	Yes	Yes	Yes
	87790	Brain, Heart, Liver, Kidney, Lung and Spleen Tissue	NEB + Mnase	Yes		Yes	
			PEB	Yes		No*	
Mitochondria Isolation Kit for Cultured Cells or Tissues	**89874**	NIH 3T3 and C6 cells		Yes	Yes	Yes	Yes
	89801	Heart and Liver Tissue		Yes	Yes	Yes	Yes
Lysosome Enrichment Kit for Tissues and Cultured Cells	**89839**	Liver and Kidney Tissue and HeLa and A431cells			Yes		

** need compatibility reagent*

DOWNSTREAM COMPATIBILITY					
Immunoprecipitation (IP)	**Immunoassays (Western Blot, ELISA)**	**EMSA (Gel Shift Assays)**	**Reporter Assays**	**Amine-reactive Protein Labeling**	**Other**
Yes	Yes	Yes	NA	Yes	Kinase Assays, Enzyme Assays and Protein Purification
Yes	Yes	Yes	Yes	Yes	Enzyme Assays and Protein Purification
Not recommended	Yes			No	Protein Purification
Yes	Yes		Yes	No	
Yes	Yes			Yes	Enzyme Assays and Pulldown Assays
Yes	Yes			Yes	Enzyme Assays
Yes	Yes	Yes	Yes	Yes	RNA EMSA, Kinase and Enzyme Assays, RT-PCR
	Yes	Yes	Yes	Yes	Enzyme Assays
Yes	Yes			Yes	
Yes	Yes			Yes	
	Yes			N/A	
	Yes	Yes	Yes	Yes	Enzyme Assays
	Yes	N/A	Yes	Yes	Enzyme Assays
	Yes	Yes	Yes	Yes	Enzyme Assays
	Yes	No	Yes	No	Enzyme Assays
	Yes	N/A	Yes	No	
	Yes			Yes	Apoptosis, Signal Transduction and Metabolic Studies
	Yes			Yes	Apoptosis, Signal Transduction and Metabolic Studies
	Yes			Yes	Signal Transduction

▸ Learn more at thermofisher.com/proteinextraction

Thermo Scientific Pierce Primary Cardiomyocyte Isolation Kit

Easily isolate and culture functional primary cardiomyocytes.

The Thermo Scientific™ Pierce™ Primary Cardiomyocyte Isolation Kit provides validated reagents, tissue-specific dissociation enzymes and an optimized, time-saving protocol for isolating and culturing primary cardiomyocytes from neonatal mouse/rat hearts. The isolated primary cardiomyocytes express cardiomyocyte protein markers and exhibit contractile function (beating activity), thereby providing a model system for studies of contraction, ischaemia, hypoxia and the toxicity of various compounds.

Highlights:

- **Optimized** – procedure and reagents optimized for viability, yield, purity and ease of use
- **Time-saving** – isolation protocol requires less than two hours compared to up to 21 hours using other methods
- **Yield** – provides a 1.5- to 4-fold increase in yield compared to do-it-yourself methods and other commercial kits
- **Viability** – provides higher viability than do-it-yourself methods and other commercial kits
- **Functional** – cultured cardiomyocytes express the appropriate biochemical markers with validated beating activity

Applications:

- Cardiomyocyte cell differentiation
- IHC
- Functional and biochemical assays
- Preclinical drug discovery and predictive disease modeling

Current methods for dissociation of cardiac cells from heart tissue require repeated (five to eight incubations) or lengthy (> 16 hours) enzyme digestion, resulting in reduced yield and viability of isolated cells. The Pierce Primary Cardiomyocyte Isolation Kit provides fully optimized reagents and a protocol that prevents over-digestion and potential damage to the isolated cells. The complete process from handling primary tissues to seeding cells in culture vessels can be completed within two hours.

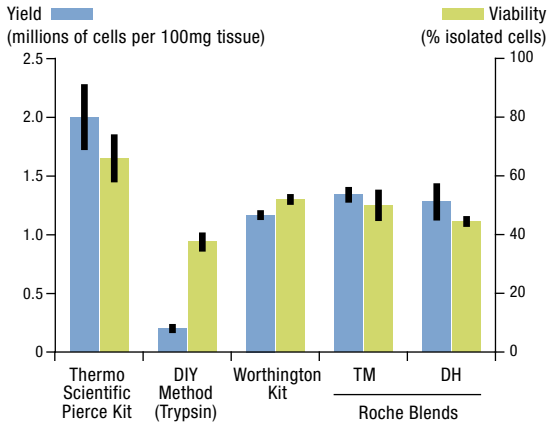


Figure 1. Cell yield and viability after cell isolation with the Thermo Scientific Pierce Primary Cardiomyocyte Isolation Kit. Mouse neonatal hearts at Day 2 were minced and incubated with Thermo Scientific™ Pierce™ Cardiomyocyte Isolation Enzymes 1 and 2 for 35 minutes or incubated with the indicated enzymes according to the manufacturers' instructions. Tissue was disrupted by pipetting up and down 25 times to generate a single-cell suspension. Cell viability was determined by trypan blue exclusion assay and total cell yield was determined using an Invitrogen™ Countess™ Automated Cell Counter.

Table 1. Cell yield and viability from typical isolations with the Thermo Scientific Pierce Cardiomyocyte Isolation Kit.

Cell Type	Yield per mL	Viability*
Mouse cardiomyocytes	2.0×10^6	63%
Rat cardiomyocytes	2.5×10^6	62%

**Viability was determined by trypan blue exclusion.*

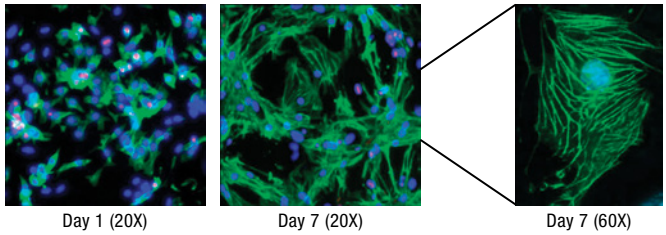


Figure 2. Cardiomyocyte differentiation after cell isolation. Mouse neonatal cardiomyocytes were isolated and cultured using the Pierce Primary Cardiomyocyte Isolation Kit. Cardiomyocytes were grown on a 24-well plate at a density of 5 x 10^5 cells/well or a 35mm glass bottom plate at a density of 2.5 x 10^6 cells/plate. At Day 1 and Day 7, cells were fixed with 4% paraformaldehyde, permeabilized and stained with propidium iodide (pink) and Troponin T Cardio Isoform (green, Product # MA5-12960). Nuclei were visualized using Hoechst™ 33342 Stain (blue, Product # 62249). Images were taken at 20X and 60X. The magnified image indicates a striated pattern, indicative of differentiated and functional cardiomyocytes.

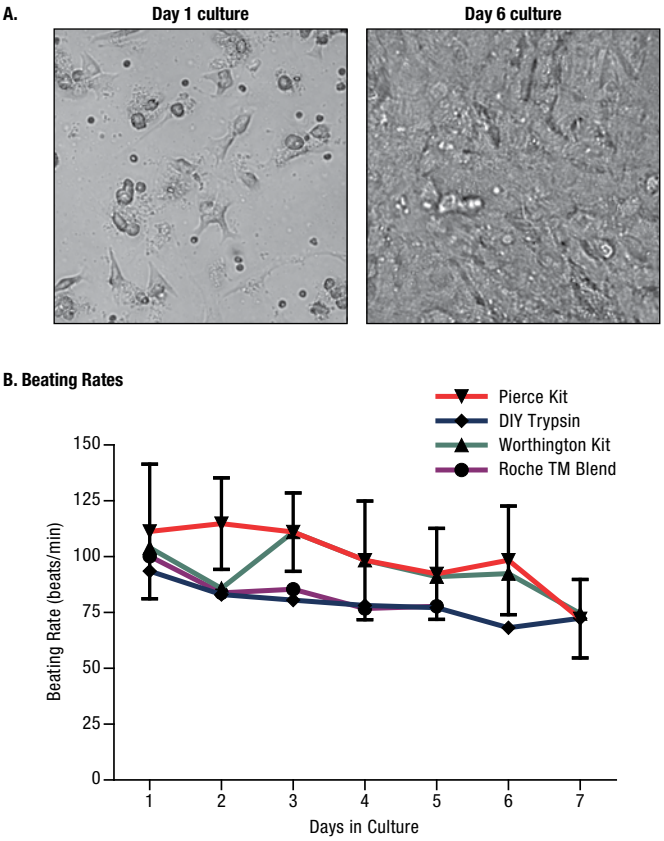


Figure 3. Beating of isolated mouse cardiomyocytes as an indication of function. A. Day 1 and Day 6 cultures of isolated mouse cardiomyocytes. **B.** Beat rates of mouse cardiomyocytes from three random fields were measured and averaged on the indicated days. Data represent the mean ± SD. Error bars were of similar sizes for all four series, but only those for the Pierce Kit are displayed.

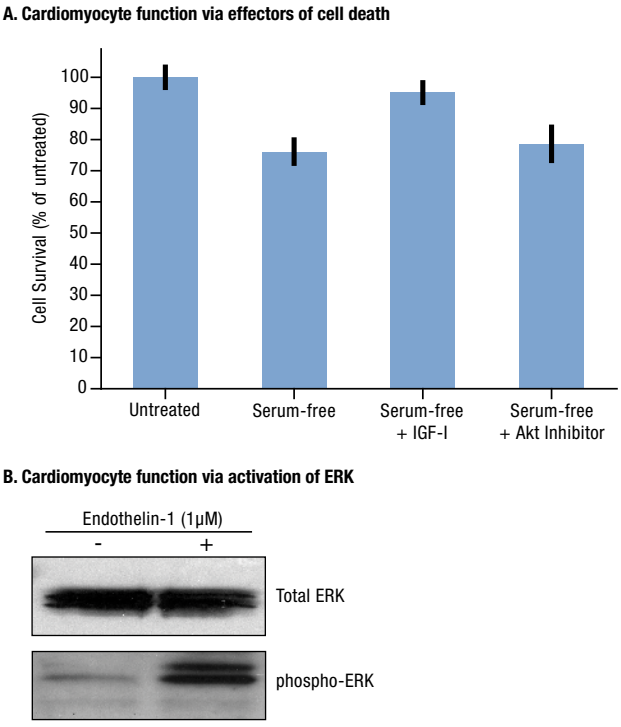


Figure 4. Example experiments with isolated cardiomyocytes. A. IGF-I mediates cardiomyocyte survival in culture in an Akt inhibitor-sensitive manner. Mouse cardiomyocytes at Day 5 in culture were incubated with or without 50ng/mL IGF-I under serum-deprivation conditions for 24 hours, and cell viability was evaluated by PI staining. A total of 200 cells were analyzed from two independent experiments. Data represent the mean ± SD. **B.** Endothelin-1 induced MAP kinase activation. Neonatal mouse cardiomyocytes were stimulated with Endothelin-1 (1µM for 24 hours), cardiomyocytes were lysed, and the phosphorylation of ERK was determined by Western blot analysis using a specific phospho-ERK antibody.

References

1. Louch, W.E. (2011). *J. Mol. Cell Cardiol.* **51(3)**:288-298.
2. Chlopcikova, S., *et al.* (2001). *Biomed. Papers* **145(2)**:49-55.
3. Gorelik, J., *et al.* (2004). *Int. J. Obstet. Gynaecol.* **111**:867-70.
4. Yoshida,T. (2014). J. *Biol. Chem.* **289:**26107-26118.

Ordering Information

Product #	Description	Pkg. Size
88281	**Pierce Primary Cardiomyocyte Isolation Kit** *Sufficient reagents to isolate cardiomyocytes from 50 neonatal mouse/rat hearts. Also contains reagents that support the culture of cardiomyocytes.* Includes: DMEM for Primary Cell Isolation, Hanks' Balanced Salt Solution (HBSS without Ca^{2+}/Mg^{2+}) Pierce Cardiomyocyte Isolation Enzyme 1 (with papain), lyophilized Pierce Cardiomyocyte Isolation Enzyme 2 (with thermolysin), 100µL Cardiomyocyte Growth Supplement (1000X)	1 kit 500mL 500mL 5 vials 5 vials 0.5mL
88287	**DMEM for Primary Cell Isolation**	500mL
88284	**Hanks' Balanced Salt Solution (HBSS, without Ca^{2+}/Mg^{2+})**	500mL
88288	**Pierce Cardiomyocyte Isolation Enzyme 1 (with papain)**	1 vial
88289	**Pierce Cardiomyocyte Isolation Enzyme 2 (with thermolysin)**	100µL

Thermo Scientific Pierce Primary Neuron Isolation Kit

Easily isolate and culture highly viable functional primary neurons.

The Thermo Scientific™ Pierce™ Primary Neuron Isolation Kit provides a simple, reliable and convenient method for the isolation and culture of primary neurons from embryonic mouse/rat cerebral cortex or hippocampus. The kit consists of tissue-specific dissociation reagents and a validated protocol to ensure a high yield of viable and functional neurons when used by both experienced and non-experienced users. The fully optimized culture reagents are designed to provide optimal growth conditions for maintaining highly pure primary neurons in either short- or long-term cultures.

The Pierce Primary Neuron Isolation Kit has been optimized to provide excellent cell yield and to sustain cells at greater than 95% viability. The neurons are appropriately polarized, develop extensive axonal and dendritic arbors, express neuronal and synaptic markers, and form numerous, functional synaptic connections with one another. They can be used as a model system for molecular and cellular biology studies of neuronal development and function, especially for visualizing the subcellular localization of endogenous or expressed proteins, neuronal polarity, and dendritic growth and synapse formation.[1]

Highlights:

- **Optimized** – for viability, yield, purity and ease of use
- **Yield** – provides a 2-fold increase in yield compared to do-it-yourself methods
- **Viability** – greater than 95% of isolated cells remain viable in culture from Day 1 to Week 4
- **Functional** – cultured neurons develop processes indicative of complete differentiation and functionality

Applications:

- Neuronal cell differentiation
- IHC
- Functional and biochemical assays
- Preclinical drug discovery, neurotoxicity testing and predictive disease modeling

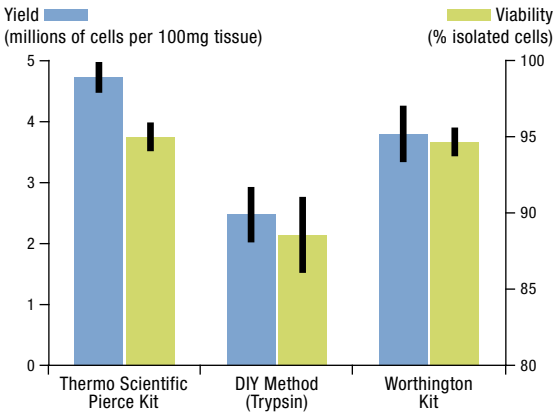


Figure 1. Cell yield and viability comparison. Mouse embryonic cortical tissue at E16-19 was incubated with Thermo Scientific™ Pierce™ Neuronal Isolation Enzyme for 30 minutes according to the literature or manufacturer's protocol. Tissue was disrupted by pipetting tip (20X) to generate a single-cell suspension. Cell viability was determined by trypan blue exclusion assay and total cell yield was determined using an Invitrogen Countess Automated Cell Counter.

Table 1. Cell yield and viability from a typical isolation with the Thermo Scientific Pierce Primary Neuron Isolation Kit. Results are for one pair of cortices or three pairs of hippocampi in 1.5mL cell suspension. Viability was determined by trypan blue exclusion.

Cell Type	Yield (cells/mL)	Viability (%)
Mouse cortical neuron	4.5×10^6	95%
Mouse hippocampal neuron	3.6×10^6	95%
Rat cortical neuron	4.0×10^6	96%
Rat hippocampal neuron	4.0×10^6	97%

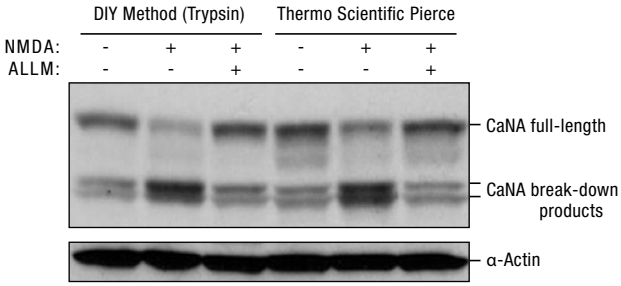
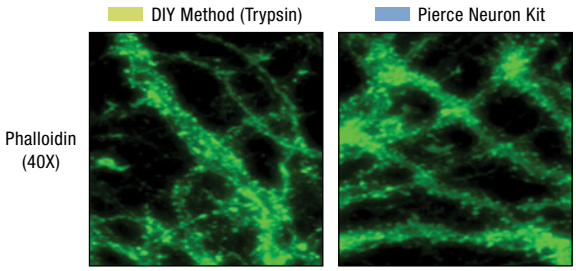


Figure 2. Synaptic protein changes in response to an NMDA-induced neuroexcitotoxic model. Cultured neurons at Day 15 were incubated with 500µM NMDA for 2 hours. Where indicated, the calpain inhibitor II (ALLM, 25µM) was added 30 minutes before NMDA stimulation. Neurons were collected 2 hours after NMDA stimulation and samples were subjected to Western blot analysis to detect changes in CaNA. β-Actin was used as a loading control.

A. Phalloidin labeling, day 21



B,C. Sholl analysis

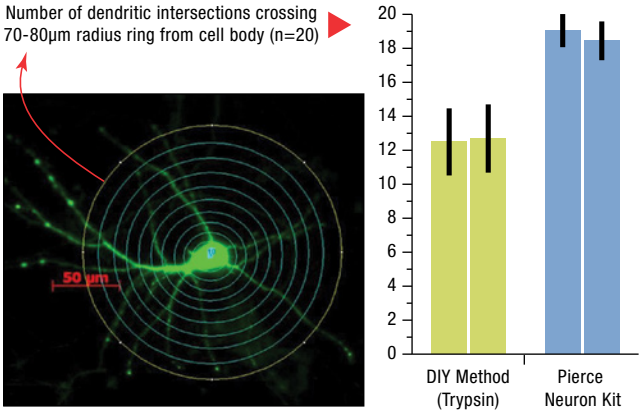


Figure 3. Dendritic complexity measurements of primary neuron cultures.
A. Representative images of dendritic segments labeled with Phalloidin at Day 21 in culture.
B. Representative illustration of an application of Sholl analysis to a GFP-labeled neuron.
C. Sholl analysis of total number of dendritic intersection crossing 70-80µm radius ring from cell body (n=20 neurons).

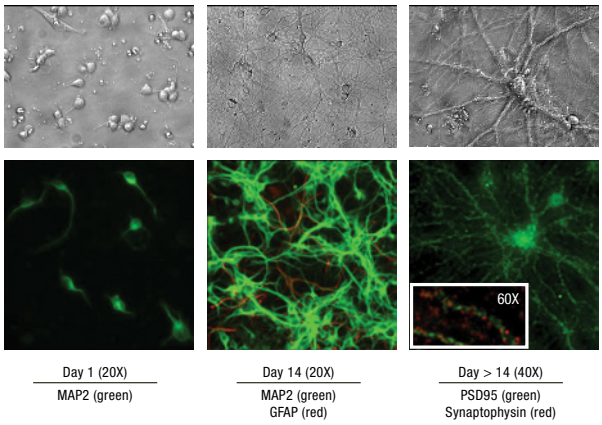


Figure 4. Primary cortical neuron cultures at different developmental stages in culture. Cortical neurons were grown on 24-well plates at a density of 1 x 10^5 cells/well or a 35mm glass bottom plate at a density of 5 x 10^6 cells/plate. **Upper panel:** Phase-contrast images of mouse cerebral cortical cultures after 1, 14 and 28 days. **Lower panel:** Immunostaining of cortical neurons at Day 1 and Day 14 with neuronal protein markers. Cells were fixed with 4% paraformaldehyde, permeabilized and stained with microtubule-associated protein 2 (MAP2, green, Product # 1861751), glial fibrillary acidic protein (GFAP, red), postsynaptic density protein 95 (PSD95, green) and synaptophysin (red). Images were taken at 20X, 40X or 60X. The insert image indicates positive staining of synaptophysin and PSD95 at synaptic terminals, indicative of differentiated and functional neurons.

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Ordering Information

Product #	Description	Pkg. Size
88280	**Pierce Primary Neuron Isolation Kit** *Sufficient reagents to isolate primary neurons from 50 pairs of embryonic mouse/rat cortexes or 150 pairs of embryonic mouse/rat hippocampi. Also contains reagents to support the culture of primary cortical and hippocampal neurons.* Includes: Neuronal Culture Medium Hanks' Balanced Salt Solution (HBSS without Ca^{2+}/Mg^{2+}) Pierce Neuronal Isolation Enzyme (with papain), lyophilized Neuronal Culture Medium Supplement (50X) Glutamine Supplement (100X) Neuronal Growth Supplement (1000X)	1 kit 500mL 500mL 5 vials 10mL 5mL 0.5mL
88283	**Neuronal Culture Medium**	500mL
88284	**Hanks' Balanced Salt Solution (HBSS, without Ca^{2+}/Mg^{2+})**	500mL
88285	**Pierce Neuronal Isolation Enzyme (with papain)**	1 vial
88286	**Neuronal Culture Media Supplement**	10mL

Thermo Scientific Pierce Mouse Embryonic Fibroblast Isolation Kit

Easily isolate and culture highly viable mouse embryonic fibroblasts.

The Thermo Scientific™ Pierce™ Mouse Embryonic Fibroblast (MEF) Isolation Kit provides validated reagents and an optimized procedure for isolating and culturing fibroblasts from mouse embryos. The kit contains a tissue-specific dissociation enzyme, culture medium and a validated protocol that enables isolation of MEFs with high viability and purity.

Highlights:

- **Optimized** – procedure and reagents optimized for viability, yield and ease of use
- **Yield** – provides a 2-fold increase in yield compared to do-it-yourself methods and other commercial kits
- **Functional** – cultured fibroblasts express the appropriate biochemical markers

Applications:

- Generation of immortalized cell lines from transgenic mice
- Feeder layer for stem cells
- IHC and IF studies

MEFs isolated and cultured using the Pierce MEF Isolation Kit can serve as a cell culture model for a diverse range of studies such as gene regulation and stem cell research.[1,2,3,4] MEFs are used most commonly as feeder layers to maintain mouse embryonic stem cells in an undifferentiated state. With a combination of transcription factors, MEFs can be converted to a pluripotent state or directly reprogrammed to various cell types such as functional neurons and cardiomyocytes, which could have important implications for studies of development, neurological and cardiac disease modeling, drug discovery, and regenerative medicine.[3,5,6] The Pierce MEF Isolation Kit also can be used to isolate MEFs from different genetically altered mouse models to study growth control and DNA damage response.[7]

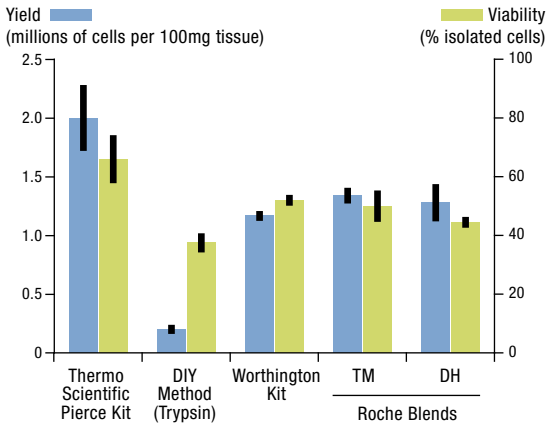

Figure 1. Cell yield and viability comparison after cell isolation using the Thermo Scientific Pierce Mouse Embryonic Fibroblast Isolation Kit. Mouse embryonic tissue at E11-13 from one mouse embryo was minced, incubated with Thermo Scientific™ Pierce™ Mouse Embryonic Fibroblast Isolation Enzyme or incubated with trypsin for 30 minutes. Tissue was disrupted by pipetting (20X) to generate a single-cell suspension. Cell viability was determined by trypan blue exclusion assay and total cell yield was determined using an Invitrogen Countess Automated Cell Counter.

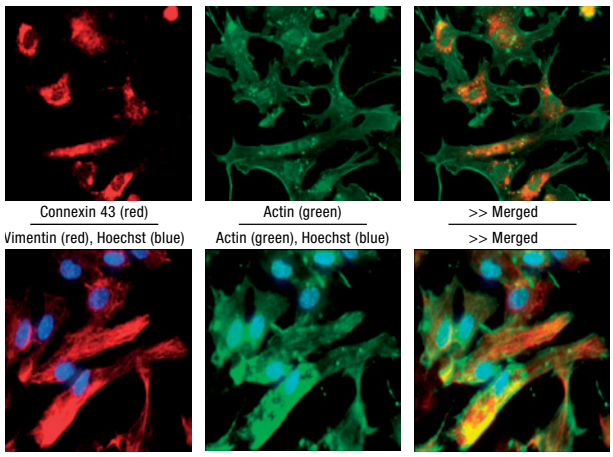

Figure 2. Representative images of MEFs immunostained with specific protein markers. Fibroblasts were grown in 24-well plates seeded at 1 x 10^5 cells/well. At Day 7, cells were fixed with 4% paraformaldehyde, permeabilized and stained with antibodies specific to Connexin 43 (red, upper panel, Product # PA1-37497) or Vimentin (red, lower panel) and β-Actin (green). Nuclei (lower panels only) were visualized using Hoechst 33342 Stain (blue, Product # 62249). Images were taken at 40X.

References

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Ordering Information

Product #	Description	Pkg. Size
88279	**Pierce Mouse Embryonic Fibroblast (MEF) Isolation Kit** *Sufficient reagents to isolate MEFs from 50 embryonic mouse somatic tissues.* Includes: DMEM for Primary Cell Isolation Pierce MEF Isolation Enzyme (with papain), Hanks' Balanced Salt Solution (HBSS without Ca^{2+}/Mg^{2+})	1 kit 500mL 5 vials 500mL
88287	**DMEM for Primary Cell Isolation**	500mL
88284	**Hanks' Balanced Salt Solution (HBSS, without Ca^{2+}/Mg^{2+})**	500mL
88290	**Pierce Mouse Embryonic Fibroblast Isolation Enzyme (with papain)**	1 vial

Thermo Scientific T-PER Tissue Protein Extraction Reagent

Mild solution designed for total protein extraction from tissue samples.

Thermo Scientific™ T-PER™ Tissue Protein Extraction Reagent uses a proprietary detergent in 25mM bicine, 150mM sodium chloride (pH 7.6) to maximize the efficiency of protein solubilization from mammalian tissue samples by homogenization. The simple composition of this reagent is compatible with additives such as protease inhibitors, salts, reducing agents and chelating agents, providing versatility for many different sample types and lysis applications. Cell lysates prepared with T-PER Reagent are directly compatible with reporter assays (e.g., luciferase, β-galactosidase, chloramphenicol acetyl transferase), protein kinase assays (e.g., PKA, PKC, tyrosine kinase), immunoassays (e.g., Western blots, ELISAs, RIAs) and/or protein purification procedures.

Highlights:

- **Simple procedure** – homogenize tissue sample in 1:20 (w/v) of tissue to T-PER Reagent, then centrifuge to pellet cell/tissue debris
- **Easy to use** – mild detergent is dialyzable for quick and easy removal
- **Versatile** – can be used with additional components (e.g., protease inhibitors, salts, reducing agents, chelating agents, etc.)[1-5]
- **Compatible** – the lysate is compatible with standard protein assays such as Thermo Scientific™ Pierce™ Coomassie Plus (Bradford) Protein Assay (Product # 23236) and Thermo Scientific™ Pierce™ 600nm Protein Assay (Product # 22660)[1-5] and may be used for reporter assays, protein kinase assays, immunoassays, ELISAs, Western blots and protein purifications[1-5]
- **Robust** – validated for yield and extraction efficiency in heart, liver, kidney, lung and spleen tissues

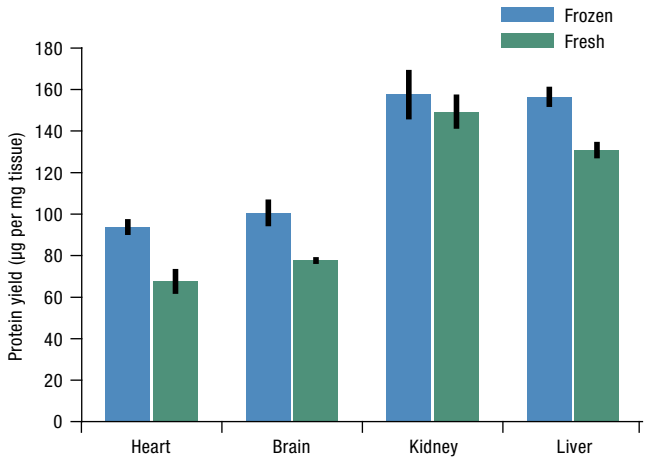

Figure 1. Protein yields from various tissues using Thermo Scientific T-PER Tissue Protein Extraction Reagent. Duplicate tissue samples were weighed, resuspended in 1:10 to 1:20 w/v T-PER Reagent and disrupted in a chilled Dounce or benchtop tissue homogenizer. The resulting lysates were centrifuged at 10,000 *x g* for 5 minutes and the supernatant was collected. The protein concentration of each lysate was determined using the Thermo Scientific™ Pierce™ BCA Protein Assay (Product # 23227) to determine protein yield per milligram of starting tissue.

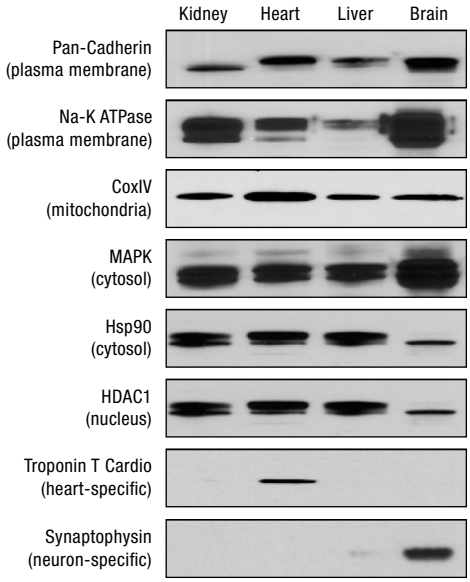

Figure 2. Protein extraction from cellular compartments using Thermo Scientific T-PER Tissue Protein Extraction Reagent. Lysates were prepared using T-PER Reagent and extraction efficiency from the various cellular compartments of different tissues compared. For each target protein, 10µg of lysate was loaded and electrophoresed by SDS-PAGE, transferred to nitrocellulose membrane and detected by Western blot using Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate (Product # 34080).

References

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Ordering Information

Product #	Description	Pkg. Size
78510	**T-PER Tissue Protein Extraction Reagent** *Sufficient for 25g fresh tissue.*	500mL

Thermo Scientific M-PER Mammalian Protein Extraction Reagent

Gentle formulation designed for total protein extraction from mammalian cells.

Thermo Scientific™ M-PER™ Mammalian Protein Extraction Reagent is designed to provide highly efficient total soluble protein extraction from cultured mammalian cells. M-PER Reagent is a nondenaturing detergent formulation that dissolves cell membranes and extracts total soluble cellular protein in only 5 minutes. M-PER Reagent requires little or no mechanical disruption, does not denature proteins and is compatible with downstream assays.

Highlights:

- **Gentle** – mild detergent lysis, yielding extracts that are immediately compatible with Coomassie (Bradford), Pierce BCA and Pierce 660nm Protein Assays or SDS-PAGE[1]
- **Compatible** – extracts soluble proteins in nondenatured state, enabling direct use in immunoprecipitation and other affinity purification procedures
- **Easy to use** – amine-free and fully dialyzable formulation enables compatibility with subsequent assay systems
- **Convenient** – lyse adherent cells directly in plate or after harvesting and washing in suspension
- **Recover active protein** – maintain luciferase, β-galactosidase, chloramphenicol acetyltransferase (CAT) and other reporter gene activities as well or better than competitor products and freeze/thaw methods
- **Robust** – validated for yield and extraction efficiency in HeLa, NIH 3T3, Jurkat and FM2 cultured mammalian cells and in primary cardiomyocytes and embryonic fibroblasts

M-PER Reagent is compatible with (A) luciferase, (B) β-galactosidase and (C) CAT assays, three popular gene regulation reporter assays (Figure 3). Compared to lysing with another supplier's lysis buffer followed by one freeze/thaw cycle (as suggested by the manufacturer) or the standard freeze/thaw method, the use of M-PER Reagent resulted in equivalent or higher enzyme activity.

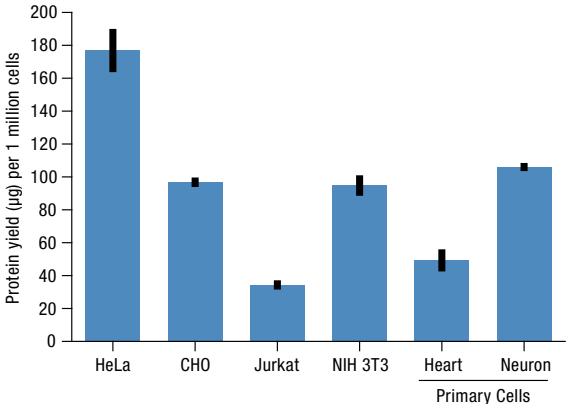

Figure 1. Protein yield from various cell types using Thermo Scientific M-PER Mammalian Protein Extraction Reagent. Cells were harvested at 85% confluency, washed twice and collected in ice-cold PBS and counted. For each cell type, 1 x 10^6 cells were pelleted by centrifugation at 2000 x *g* for 5 minutes and lysed in 1mL M-PER Reagent for 5 minutes. The cell lysates were clarified by centrifugation at 14,000 x *g* for 10 minutes, the supernatant was collected and the protein concentration (µg/million cells) was determined using the Pierce BCA Protein Assay (Product # 23227).

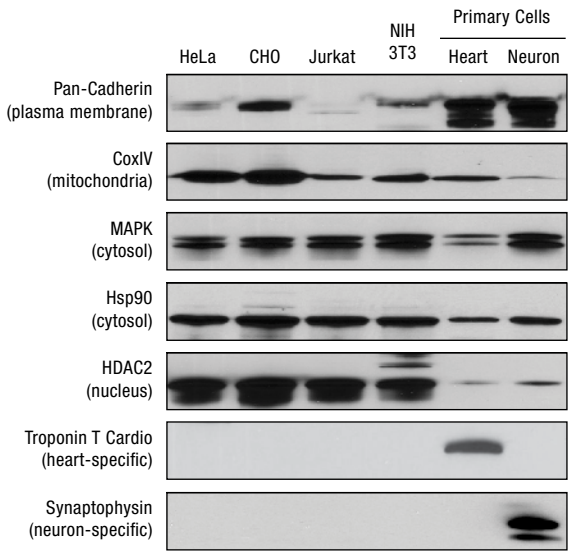

Figure 2. Protein extraction efficiency from major cellular compartments using Thermo Scientific M-PER Mammalian Protein Extraction Reagent. Lysates from established cell lines and primary cultures were prepared using M-PER Reagent and extraction efficiency from the various cellular compartments evaluated. For each target protein, 10µg of lysate was loaded and electrophoresed by SDS-PAGE, transferred to nitrocellulose membrane and detected by Western blot using SuperSignal West Pico Chemiluminescent Substrate (Product # 34080).

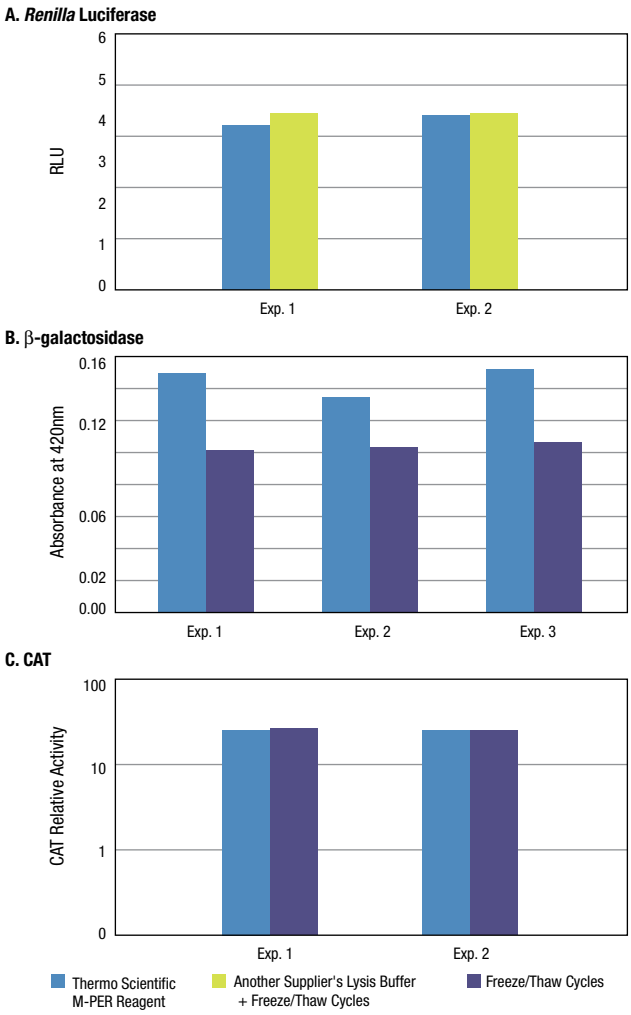

Figure 3. Thermo Scientific M-PER Reagent compatibility with reporter assays in transiently transfected mammalian cells. Mammalian FM2 cells were transiently transfected with a luciferase reporter construct. The transfected cells were lysed with either M-PER Reagent or another supplier's lysis buffer and assayed for luciferase activity (Panel A). For β-galactosidase and CAT assays, MDA-MB-231 cells were cotransfected with reporter constructs expressing β-galactosidase and CAT, respectively. The transfected cells were lysed with M-PER Reagent or the freeze/thaw method, and the lysates were assayed for β-galactosidase and CAT activity (Panels B and C).

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Ordering Information

Product #	Description	Pkg. Size
78503	**M-PER Mammalian Protein Extraction Reagent** *Sufficient for 2.5g of wet cells (10 million cells per 1mL of reagent).*	25mL
78501	**M-PER Mammalian Protein Extraction Reagent** *Sufficient for 25g of wet cells (10 million cells per 1mL of reagent).*	250mL
78505	**M-PER Mammalian Protein Extraction Reagent** *Sufficient for 100g of wet cells (10 million cells per 1mL of reagent).*	1L

Thermo Scientific Pierce RIPA Buffer

High-performance buffer for cell lysis and protein extraction.

The Thermo Scientific™ Pierce™ RIPA Buffer is used to lyse cultured mammalian cells, including plated cells and cells pelleted from suspension cultures. It enables the extraction of membrane, nuclear and cytoplasmic proteins and is compatible with many applications, including reporter assays, the Pierce BCA Protein Assay, immunoassays and protein purification. Inhibitors such as Thermo Scientific™ Protease Inhibitor Cocktails and Tablets and Phosphatase Inhibitor Cocktails and Tablets are also compatible with Pierce RIPA Buffer and can be added before use to prevent proteolysis and maintain protein phosphorylation.

Highlights:

- **Convenient** – ready-to-use solution
- **Flexible** – compatible with many applications, including protein assays, immunoassays and protein purification
- **Versatile** – enables extraction of cytoplasmic, membrane and nuclear proteins
- **Disclosed formulation** – contains no proprietary components
- **Validated in** – HeLa and A431 cultured mammalian cells

Pierce RIPA Buffer derives its name from the original application for which it was developed: the radio-immunoprecipitation assay. While this isotopic assay method is rarely performed in laboratories today, the acronym for this lysis buffer formulation has endured in common use. Pierce RIPA Buffer is highly effective for protein extraction from a variety of cell types because it contains three nonionic and ionic detergents. However, the RIPA formulation is not compatible with certain downstream applications compared to other lysis reagents.

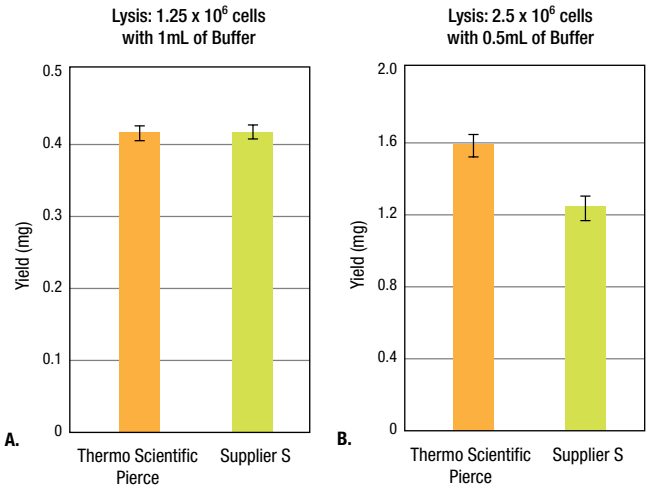

Figure 1. The Thermo Scientific Pierce RIPA Buffer extracts equivalent or more protein than the Supplier S RIPA Buffer. Pierce and Supplier S RIPA Buffer at volumes of 1 and 0.5mL were added separately to 1.25 x 10^6 and 2.5 x 10^6 HeLa cells, respectively. Cells were thoroughly resuspended and incubated for 10-15 minutes on ice with occasional swirling of tubes. After clarification of cell lysates by centrifugation, protein extraction was determined using the Pierce BCA Protein Assay Kit (Product # 23225). Protein extraction was equal when 1.25 x 10^6 cells were lysed with 1mL of buffer (Figure 1A), but the Pierce RIPA Buffer extracted more protein than Supplier S's buffer when 2.5 x 10^6 cells were lysed with only 0.5mL (Figure 1B).

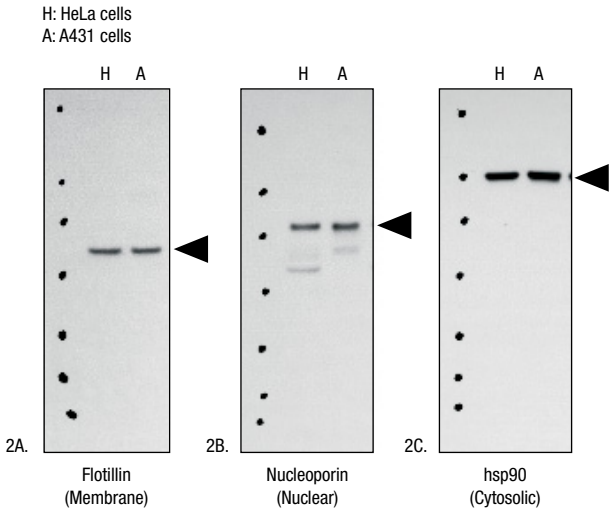

Figure 2. Isolation of membrane, nuclear and cytosolic proteins using Thermo Scientific Pierce RIPA Buffer. The Pierce RIPA Buffer extracts proteins from membrane **(2A)**, nuclear **(2B)** and cytosolic **(2C)** fractions. Pierce RIPA Buffer was supplemented with Thermo Scientific™ Halt™ Protease Inhibitor Cocktail (Product # 78410) and used to lyse HeLa and A431 cells. Western blotting was performed using mouse anti-flotillin, -nucleoporin and -hsp90 antibodies (BD Biosciences) at 0.25µg/mL, 1µg/mL and 0.25µg/mL, respectively, and goat anti-mouse-HRP (20ng/mL, Product # 31430). The signal was detected using SuperSignal West Pico Chemiluminescent Substrate (Product # 34080).

References

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Ordering Information

Product #	Description	Pkg. Size
89900	**Pierce RIPA Buffer** *Sufficient for approx. 5 million cells per 1mL of reagent.*	100mL
89901	**Pierce RIPA Buffer** *Sufficient for approx. 5 million cells per 1mL of reagent.*	250mL

Thermo Scientific Pierce IP Lysis Buffer

Optimized for yield and compatibility with immunoprecipitation (IP).

The Thermo Scientific™ Pierce™ IP Lysis Buffer is optimized for cell lysate yield and compatibility with downstream immunoprecipitation (IP) and co-immunoprecipitation (co-IP) assays.

Pierce IP Lysis Buffer is a mammalian whole cell lysis reagent based on a modified RIPA buffer formulation without SDS. This moderate-strength lysis buffer effectively solubilizes cellular proteins but does not liberate genomic DNA or disrupt protein complexes like ordinary RIPA buffer. Pierce IP Lysis Buffer is specially formulated for pull-down and IP assays.

Highlights:

- **Compatible** – optimized for IP and pull-down assays; also compatible with protein assays, reporter assays and immunoassay procedures
- **Effective** – ready-made formula optimized for extracting cytoplasmic, membrane and nuclear proteins
- **Gentle** – helps maintain protein complexes for co-immunoprecipitation
- **Minimizes sample viscosity** – does not liberate genomic DNA, which can cause high sample viscosity
- **Validated in** – A459 cultured mammalian cells

The Pierce IP Lysis Buffer is effective for lysing cultured mammalian cells, including adherent cells and cells pelleted from suspension cultures. Optimized for pull-down and IP assays, this lysis buffer is also compatible with many other applications, including the Pierce BCA and Pierce 660nm Protein Assays, protein purification, and immunoassays (e.g., ELISA, Western blot).

The Pierce IP Lysis Buffer does not contain protease or phosphatase inhibitors; however, if desired, inhibitors such as Halt Protease Inhibitor Cocktail or Tablets or Thermo Scientific™ Halt™ Phosphatase Inhibitor Cocktail or Tablets can be added just before use to prevent proteolysis and maintain phosphorylation of proteins.

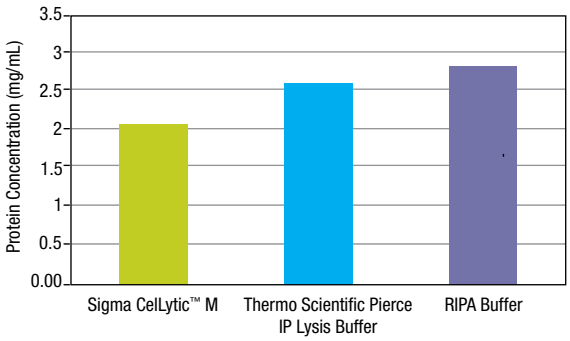

Figure 1. Protein yield obtained using Thermo Scientific Pierce IP Lysis Buffer compared to other similar formulations. A549 cells (2 x 10^6) were lysed using 200µL of three different lysis buffers, clarified by centrifugation and measured for protein concentration using the Pierce BCA Protein Assay (Product # 23227).

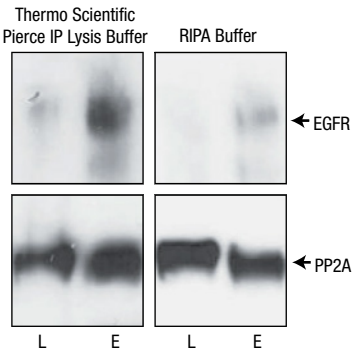

Figure 2. The Thermo Scientific Pierce IP Lysis Buffer enables highly efficient IPs. A549 cell lysates (200µg) were generated using different lysis buffers. EGFR and PP2A were immunoprecipitated using the Thermo Scientific™ Pierce™ Crosslink IP Kit (Product # 26147) and anti-EGFR antibody (Stressgen) or anti-PP2A antibody (Millipore). The IPs were washed with the same lysis buffer used to lyse the cells. Lysates representing 5% of the total load (L) and elution (E) fractions were blotted with anti-EFGR or anti-PP2A antibody and detected using SuperSignal West Dura Substrate (Product # 34076).

Figure 3. Thermo Scientific Pierce IP Lysis Buffer is compatible with immunoprecipitation. HEK293 and A431 cell lysates were prepared using Pierce IP Lysis Buffer. For IP, 500µg total cell lysate (HEK293 was used for EGFR and A431 was used for pan Akt) was incubated with 5µg biotinylated antibody (prepared using the Thermo Scientific™ Pierce™ Antibody Biotinylation Kit for IP, Product # 90407) overnight at 4°C. The immune complex was then incubated with 25µL Thermo Scientific™ Pierce™ Streptavidin Magnetic Beads (Product # 88816) for 1 hour at room temperature. The bound complex was washed extensively and eluted with a MS elution buffer (from the Thermo Scientific™ Pierce™ MS-Compatible Magnetic IP Kit, Streptavidin, Product # 90408) or by boiling in reducing sample buffer. The samples were separated by SDS-PAGE and western analysis was performed. Blots were imaged with the Thermo Scientific™ MyECL™ Imager.

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Ordering Information

Product #	Description	Pkg. Size
87787	**Pierce IP Lysis Buffer** *Sufficient for 10,000cm² of cell monolayer or 10g of cell paste.*	100mL
87788	**Pierce IP Lysis Buffer** *Sufficient for 25,000cm² of cell monolayer or 25g of cell paste.*	250mL

Thermo Scientific N-PER Neuronal Protein Extraction Reagent

Extract active proteins from brain tissue and primary cultured neurons.

Thermo Scientific™ N-PER™ Neuronal Protein Extraction Reagent is a proprietary cell lysis reagent optimized for efficient extraction of total protein from neuronal tissue and primary cultured neurons. N-PER Reagent provides higher yields and better extraction efficiency compared to other reagents and preserves protein function.

Using N-PER Reagent, protein extraction is completed in less than 30 minutes. For tissue samples, efficient extraction requires mechanical disruption (e.g., Dounce Homogenization, Polytron Mixer) in N-PER Reagent. Typical neuronal protein yields are 70-90µg of protein per mg of brain tissue or 300µg of total protein from 10^6 primary neurons. Neuronal cell lysates prepared with the N-PER Reagent may be used in downstream enzymatic activity assays (e.g., phosphatase, kinase, ATPase assays), immunoassays (e.g., Western blots, ELISAs, RIAs) and protein purification.

Highlights:

- **Optimized** – efficient extraction of total neuronal protein, including membrane proteins from tissue or primary cultured cells
- **Gentle** – preserves protein function without compromising yield
- **Versatile** – can be supplemented with protease inhibitors, reducing or chelating agents or required cofactors
- **Compatible** – extracts are suitable for use with total protein, enzymatic and immunological assays and protein purification methods
- **Validated in** – brain tissue and primary neurons

Applications:

- Isolate functional proteins from neuronal tissues or primary neuronal cells
- Study enzymatic activity assays specific to neuronal tissue
- Generate lysates from neuronal tissues for biomarker analysis

Typical neuron structures include the cell body (soma), dendrites and an axon. The unique morphology and rich sphingolipid and cholesterol composition of neuron cell membranes creates challenges in preparing neuronal protein extracts. N-PER Neuronal Protein Extraction Reagent is specifically formulated to overcome the challenges of extracting functional native proteins from brain or other neuronal tissue and primary cultured neurons, while increasing protein yield compared to other extraction methods.

For each extraction, a ratio of 1g of tissue to 10mL of N-PER Reagent or 0.5-1mL N-PER Reagent per 10cm petri dish of primary cultured cells is recommended. Halt Protease Inhibitor Cocktail or Phosphatase Inhibitor Cocktail can be added just before use to prevent proteolysis or to offer additional protection from the high phosphatase activity normally present in brain tissue.

Table 1. Average total protein yields from neuronal tissue and primary cells.

Source	Sample Size	Reagent† Volume	Average Total Protein	Protein Concentration
Fresh Mouse Brain	1 brain hemisphere	2mL	18.3 ± 0.4mg	9.31 ± 0.2mg/mL
Primary Neuronal Cells	10^6 cells	0.5mL	0.35 ± 0.01mg	0.69 ± 0.02mg/mL

† Thermo Scientific N-PER Neuronal Protein Extraction Reagent

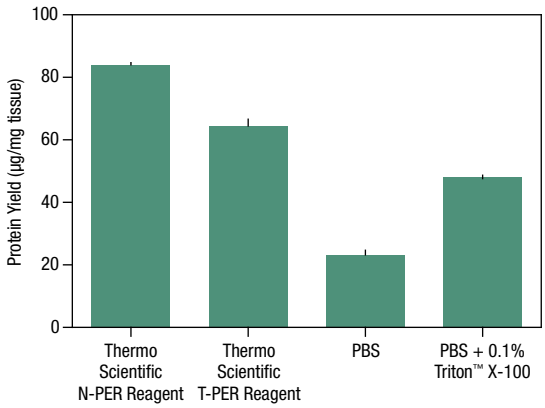


Figure 1. Increased protein yield per mg of neuronal tissue using Thermo Scientific N-PER Reagent. Yields (µg protein/mg tissue) of several extraction reagents are compared to N-PER Reagent using fresh mouse brain tissue and Dounce homogenization. All isolations were performed according to the supplied product instructions. Protein concentrations from cleared supernatants was determined using the Pierce BCA Protein Assay Kit (Product # 23225).

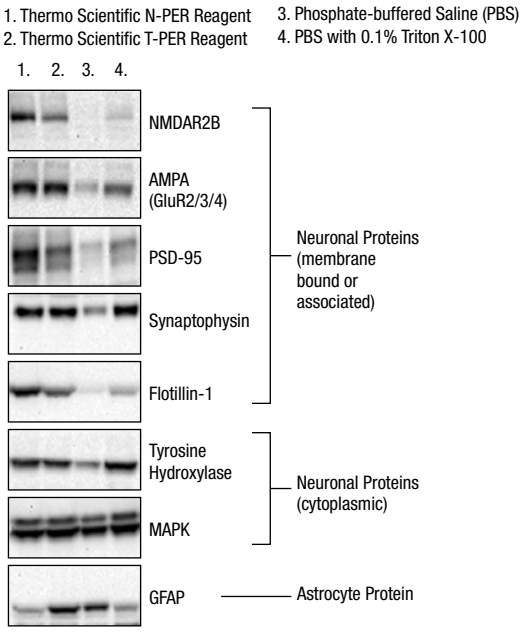


Figure 2. Increased extraction efficiency of specific neuronal proteins using Thermo Scientific N-PER Reagent. Lysates (10µL per well) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with antibodies for specific neuronal proteins, including membrane bound/associated proteins. Blots were developed with SuperSignal West Pico Chemiluminescent Substrate (Product # 34077).

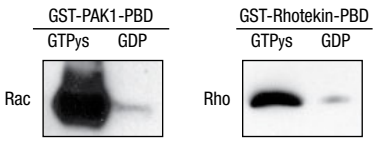


Figure 3. Thermo Scientific N-PER Reagent does not affect activity of GTPases isolated from neuronal tissue. N-PER Reagent was used to isolate brain tissue lysates. Lysates (1mg) supplemented with 5mM $MgCl_2$ and treated with GTPγS or GDP were incubated with the indicated GST-PBD and glutathione resin. Active Rho and Rac were isolated following protocols for the Active Rho Pull-down and Detection Kit (Product # 16116) and the Active Rac Pull-down and Detection Kit (Product # 16118). Half of the eluted sample volumes were analyzed by Western blot and probed using small GTPase-specific antibodies provided in the respective kits.

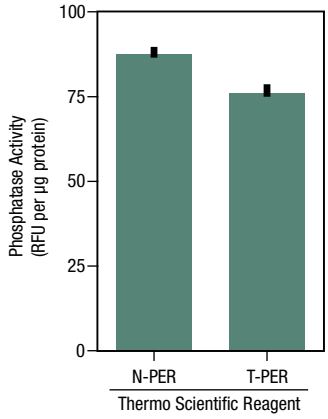


Figure 4. Protein phosphatase activity is retained after lysis and extraction. Brain tissue lysate (10mg) was incubated with a fluorogenic phosphatase substrate for 1 hour at 37°C. Subsequent hydrolysis of the substrate by phosphatases increased fluorescence.

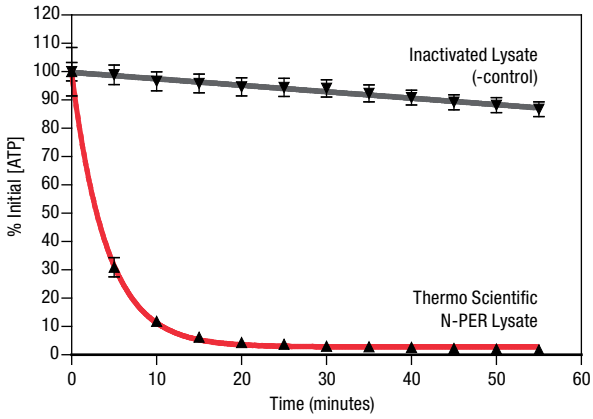


Figure 5. Kinase/ATPase retains activity after lysis and extraction. ATPase activity was measured as a function of luciferase chemiluminescence. Active kinase/ATPases hydrolyze ATP, decreasing the luminescence of luciferase.

Ordering Information

Product #	Description	Pkg. Size
87792	**N-PER Neuronal Protein Extraction Reagent** *Sufficient for 10g tissue or up to 200 x 10cm dishes of primary cultured neurons.*	100mL

Protein Extraction and Synapse Isolation from Brain Tissue and Primary Neurons

Thermo Scientific Syn-PER Synaptic Protein Extraction Reagent

Rapidly and efficiently isolate functional synaptosomal complexes.

Thermo Scientific™ Syn-PER™ Synaptic Protein Extraction Reagent is a proprietary cell lysis reagent for efficient isolation of synaptosomes containing functional synaptic proteins from neuronal tissue and primary cultured neurons.

Syn-PER Reagent is used to prepare synaptosomes containing biologically active pre-and post-synaptic proteins (i.e., intact membranes and protein complexes of synapses). When used with fresh neuronal tissue or primary cultured neurons, synaptosomes prepared with the Syn-PER Reagent can be used to study synaptic transmission. The synaptosomal proteins contained in these extracts can also be used for downstream applications such as Western blotting, immunoprecipitation, enzymatic activity assays and protein-protein interaction studies. Syn-PER Synaptic Protein Extraction Reagent minimizes the degradation of phosphoproteins and is ideal for studies requiring the preservation of phosphoprotein integrity.

Highlights:

- **Efficient synapse extraction** – obtain up to 10µg of synaptic protein per milligram of neuronal tissue or 4µg synaptic protein per 35mm dish of primary cultured neurons (10^6 cells)
- **Gentle formulation** – isolate functional synaptosomes; then lyse the synapses to extract native synaptic proteins and preserve phosphoprotein integrity
- **Fast procedure** – obtain synaptosomal suspension of intact synapses in less than one hour
- **Simple protocol** – requires no ultracentrifugation steps
- **Validated in** – brain tissue and primary neurons

Applications:

- Isolate functional synaptosomes to study neurotransmitter release
- Extract pre- and post-synaptic proteins to identify changes in protein composition and function in synapses
- Preserve and study labile or transient protein phosphorylation events associated with synapses

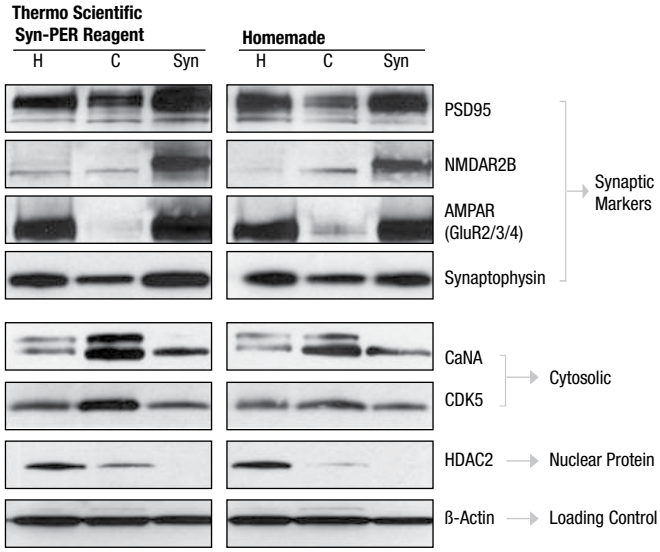
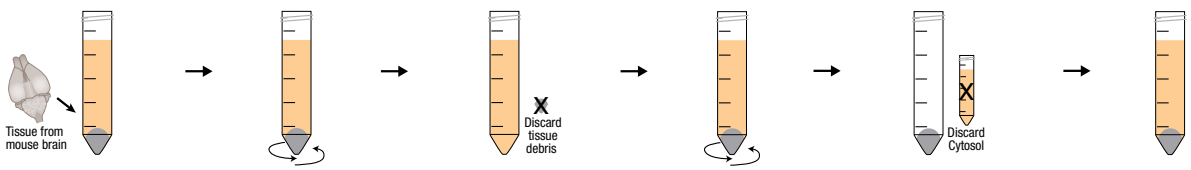


Figure 2. Improved enrichment of pre- and postsynaptic marker proteins is obtained in samples prepared using Thermo Scientific Syn-PER Reagent compared to homemade buffer. Ten micrograms total protein from homogenates (H), cytosol (C), and synaptosome suspension (Syn) were analyzed by Western blotting using antibodies against specific pre- and postsynaptic marker proteins including synaptophysin, PSD95, NMDA receptor 2B subunit, AMPA receptors (GluR2/3/4), as well as Calcineurin A (CaNA), Cdk5, HDAC2 and β-Actin as purity and loading controls. The blots were probed with goat anti-rabbit HRP or goat anti-mouse HRP and detected with SuperSignal West Pico Chemiluminescent Substrate (Product # 34077).



1. Add 10mL of Syn-PER Reagent per gram of tissue. Homogenize at 4°C.
2. Centrifuge at 1200 x *g* for 10 minutes at 4°C.
3. Discard tissue debris pellet and transfer supernatant to a new tube.
4. Centrifuge supernatant at 15,000 x *g* for 20 minutes at 4°C.
5. Remove supernatant (cytosolic fraction) from synaptosome pellet.
6. Suspend synaptosome pellet in 1-2mL of Syn-PER Reagent for downstream analysis. Maintain at 4°C (-20°C for long-term storage).

Figure 1. Protocol for the isolation of synaptosomes from mouse brain using Thermo Scientific Syn-PER Reagent or homemade buffer.

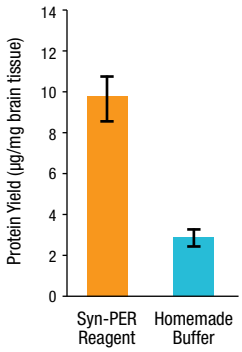


Figure 3. Comparison of protein yield from synaptosome suspensions prepared with Thermo Scientific Syn-PER Reagent and homemade buffer. Whole brain or one hemisphere excluding the cerebellums (about 200-400mg) was homogenized as one sample in 10 volumes of Syn-PER Reagent or homemade buffer (protease inhibitors included; Product # 87785) using a 7mL Dounce tissue grinder. The homogenate was centrifuged and supernatant collected. The supernatant was further centrifuged and the pellets, containing synaptosomes, were gently resuspended in their respective buffer. Protein content was estimated using the Pierce BCA Protein Assay Kit (Product # 23225).

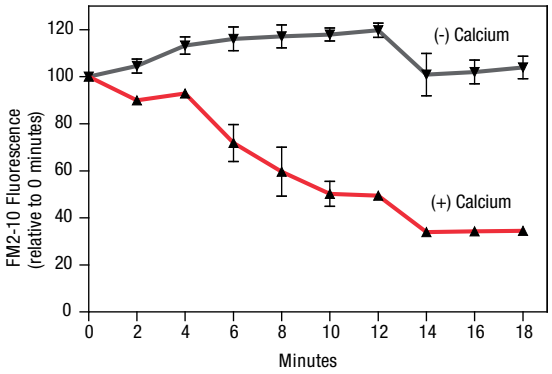


Figure 4. Ca^{2+}-dependent and KCl-evoked release of FM2-10 in synaptosomes prepared using Thermo Scientific Syn-PER Reagent. Synaptosomes were resuspended in HBSS either plus or minus 1.2mM $CaCl_2$. The suspensions were then incubated with 100µM FM2-10 for 15 minutes. The release of FM2-10 was induced by the addition of 30mM KCl. Release of accumulated FM2-10 was then monitored at Ex_{506}/Em_{620} nm as a decrease in fluorescent intensity upon release of the dye into solution where FM2-10 is no longer fluorescent. Each point is the mean ± SD of two samples.

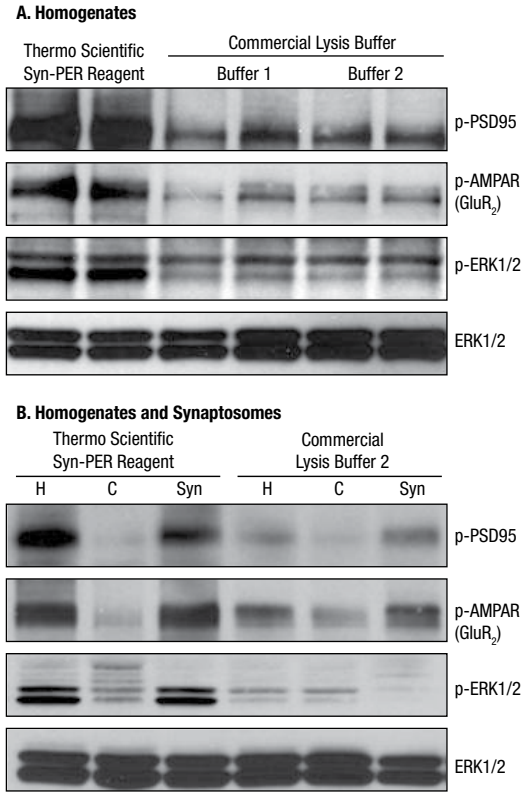


Figure 5. Thermo Scientific Syn-PER Reagent provides better preservation of phosphoprotein immunoreactivity than other commercial lysis buffers. Western blot comparison of immunoreactivity of phosphoproteins, p-PSD95, p-GluR2 of AMPA receptor, and p-ERK1/2, between samples prepared with Syn-PER Reagent and commercial lysis buffers in homogenates **(H) (Panel A)** and both homogenates, cytosol fraction **(C)** and synaptosome suspension **(Syn) (Panel B)**. Equal amounts of total protein (10–20µg/lane) were resolved on denaturing 2–10% SDS-polyacrylamide gels. Western blots were performed with the appropriate antibodies and bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Product # 34080).

References

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Ordering Information

Product #	Description	Pkg. Size
87793	**Syn-PER Synaptic Protein Extraction Reagent** *Sufficient for 10g tissue or 500 x 35mm dishes of primary cultured neurons.*	100mL

Subcellular Protein Fractionation and Organelle Isolation from Tissues and Cultured Mammalian Cells

Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Kit

A fast and easy means of obtaining concentrated nuclear extracts that can be used in a variety of downstream studies.

The Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Kit enables efficient cell lysis and extraction of separate cytoplasmic and nuclear protein fractions in less than two hours.

The NE-PER Kit is a nuclear protein extraction method that involves simple, stepwise lysis of cells and centrifugal isolation of nuclear and cytoplasmic protein fractions. A benchtop microcentrifuge, tubes and pipettors are the only tools required. The NE-PER Reagents efficiently solubilize and separate cytoplasmic and nuclear proteins into fractions with minimal cross-contamination or interference from genomic DNA and mRNA. Once desalted or diluted, the isolated proteins can be used to perform immunoassays and protein interaction experiments, such as mobility shift assays (EMSA), co-immunoprecipitation (Co-IP) and pull-down assays.

Highlights:

- **Fast** – obtain nuclear and cytoplasmic fractions of soluble proteins in less than two hours
- **Proven** – the NE-PER Kit is referenced in more than 950 distinct publications
- **Versatile** – nuclear protein extraction from either cultured cells or tissues (intended for fresh samples only)
- **Scalable** – two kit sizes for producing extracts from cells and tissues
- **Convenient** – simple instructions do not require gradient ultracentrifugation
- **Compatible** – use for downstream assays, including Western blotting, gel-shift assays, protein assays, reporter gene assays, enzyme activity assays and others[1-3]
- **Robust** – validated in heart, lung, kidney and liver tissue and HeLa, NIH 3T3, A549, C6, Cos7 and Hepa cultured mammalian cells

The preparation of good nuclear protein extracts is central to the success of many gene regulation studies. Nuclear extracts are used instead of whole cell lysates for the following reasons. First, many experiments in the area of gene regulation are adversely affected by cellular components present in whole cell lysates. Second, the concentration of the nuclear protein of interest is diluted by the vast array of cytoplasmic proteins present in whole cell extracts. Finally, whole cell lysates are complicated by the presence of genomic DNA and mRNA.

A variety of methods exist to isolate nuclei and prepare nuclear protein extracts.[4-6] However, most of these are lengthy processes requiring mechanical homogenization, freeze/thaw cycles, extensive centrifugation or dialysis steps that may compromise the integrity of many fragile nuclear proteins. The NE-PER Nuclear and Cytoplasmic Extraction Kit enables a stepwise lysis of cells that generates both functional cytoplasmic and nuclear protein fractions in less than two hours.

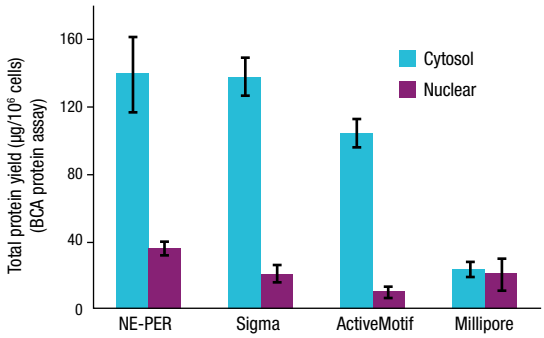

Figure 1. Protein yield comparison between different kits. HeLa cells (10^6) were extracted with the NE-PER Nuclear Protein Extraction Kit or nuclear extraction kits from other vendors. The total amount of nuclear protein obtained using the NE-PER Kit is 2- to 3.5-fold higher than with other commercially available reagents. Total protein was measured using the Pierce BCA Protein Assay.

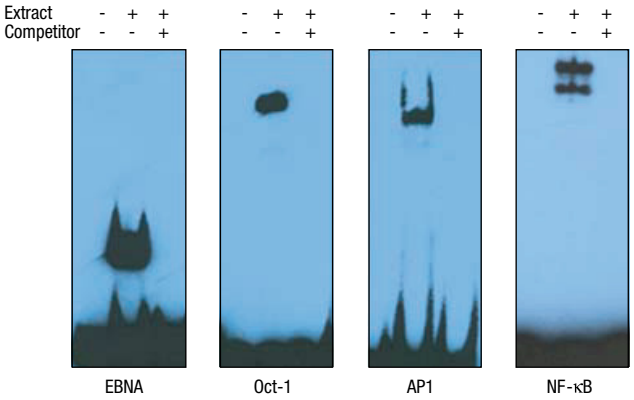

Figure 2. Chemiluminescent EMSA of four different DNA-protein complexes. DNA binding reactions were performed using 20fmol biotin-labeled DNA duplex (1 biotin per strand) and 2µL (6.8µg total protein) NE-PER Nuclear Extract prepared from HeLa cells. For reactions containing specific competitor DNA, a 200-fold molar excess of unlabeled specific duplex was used.

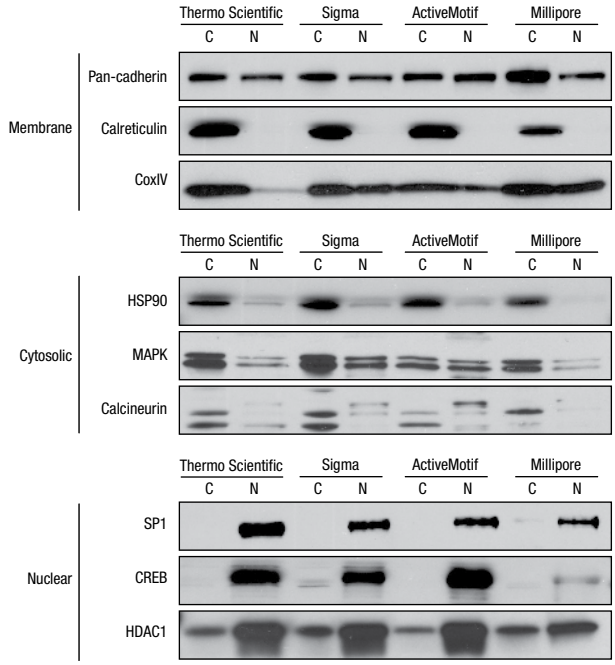

Figure 3. Nuclear and cytosolic fractions are obtained with minimal cross-contamination. HeLa cells were extracted with the NE-PER Nuclear Protein Extraction Kit or nuclear extraction kits from other vendors. Samples of the nuclear and cytosolic fractions were analyzed by Western blot using antibodies against common nuclear, cytoplasmic and membrane protein markers and visualized using SuperSignal West Pico Chemiluminescent Substrate (Product # 34080). Nuclear fractions produced with the NE-PER Kit had minimal to no contamination with cytosolic or membrane proteins, with the exception of pan-cadherin, a plasma membrane protein, which was found in the nuclear fractions of all of the preparations.

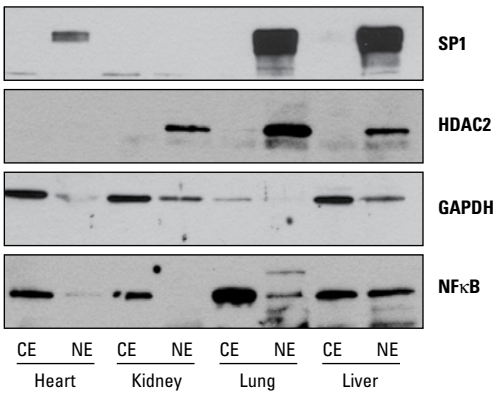

Figure 4. Western blots of specific proteins from fractionated tissues. Cytoplasmic and nuclear extract (10µg each) from different mouse tissue fractionated using the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit was analyzed by 4-20% SDS-PAGE and Western blotting. Primary antibodies specific for the target proteins were diluted 1:1,000 (SP1, HDAC2 and NFκB p65, or 1:10,000 (GAPDH). Anti-Rabbit (H+L) HRP (Product # 31460) diluted 1:25,000 was the secondary antibody and SuperSignal West Dura Chemiluminescent Substrate (Product # 34076) was used for signal detection.

References

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Ordering Information

Product #	Description	Pkg. Size
78833	**NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit** *Sufficient for extracting 50 cell pellet fractions having packed cell volumes of 20µL each (a total of ~2.0g of cell paste).* Includes: Cytoplasmic Extraction Reagent I (CER I) Cytoplasmic Extraction Reagent II (CER II) Nuclear Extraction Reagent (NER)	Kit 10mL 550µL 5mL
78835	**NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit** *Sufficient for extracting 250 cell pellet fractions having packed cell volumes of 20µL each (a total of ~10g of cell paste).* Includes: Cytoplasmic Extraction Reagent I (CER I) Cytoplasmic Extraction Reagent II (CER II) Nuclear Extraction Reagent (NER)	Kit 10mL 2.75mL 25mL

Subcellular Protein Fractionation and Organelle Isolation from Tissues and Cultured Mammalian Cells

Thermo Scientific Mem-PER Plus Membrane Protein Extraction Kit

Fast and simple enrichment of integral membrane proteins and membrane-associated proteins.

The Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit enables fast and efficient small-scale solubilization and enrichment of integral membrane proteins and membrane-associated proteins using a simple selective detergent procedure.

Traditional methods for isolating membrane proteins are tedious and time-consuming and require gradient separation and expensive ultracentrifugation equipment. The Mem-PER Plus Kit effectively isolates membrane proteins from cultured mammalian cells and tissues using a mild detergent-based, selective extraction protocol and a simple benchtop microcentrifuge procedure in less than one hour. The cells are first permeabilized with a mild detergent, allowing the release of soluble cytosolic proteins, after which a second detergent solubilizes membrane proteins. Membrane proteins with one or two transmembrane domains are typically extracted with an efficiency of up to 90%. Extraction efficiencies and yields will vary depending on cell type as well as the number of times the integral membrane protein spans the lipid bilayer. Cross-contamination of cytosolic proteins into the membrane fraction is usually less than 10%. Membrane fractions are directly compatible with many downstream applications, such as SDS-PAGE, Western blotting, BCA, immunoprecipitation and amine-reactive protein labeling techniques.

Highlights:

- **Fast and simple** – complete in approximately one hour using only a benchtop microcentrifuge
- **Flexible** – effective for both cultured mammalian cells and mammalian tissues
- **Clean preparation** – produces minimal cross-contamination of cytosolic protein (typically less than 10%)
- **Compatible** – can analyze membrane protein extracts by SDS-PAGE, Western blotting, immunoprecipitation or protein assays
- **Validated in** – heart and liver tissues and HeLa, A431, A549, C6, Cos7 and Hepa cultured mammalian cells

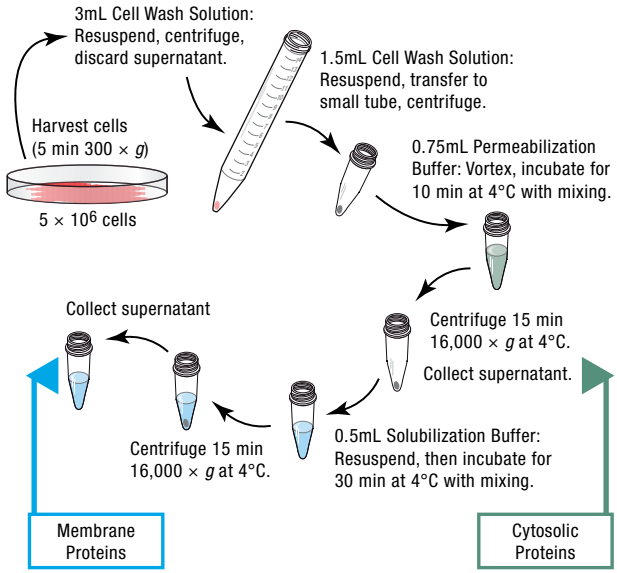

Figure 1. Thermo Scientific Mem-PER Plus Membrane Protein Extraction Kit protocol summary.

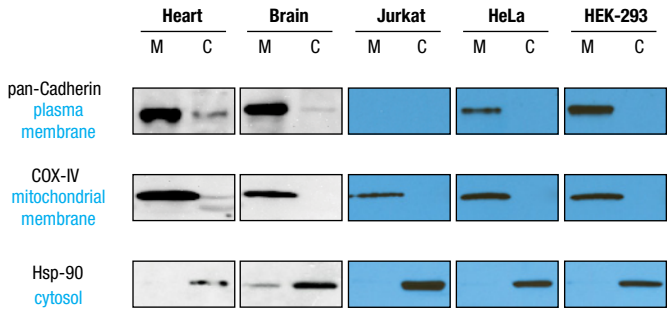

Figure 2. Efficient enrichment of membrane proteins from tissues and cell lines. Membrane proteins were isolated from 30mg of tissue (mouse brain and heart) or 5 x 10^6 cultured cells (Jurkat, HeLa or HEK-293 cell lines) using the Mem-PER Plus Membrane Protein Extraction Kit protocol. Membrane and cytosolic fractions (10mg) were separated by SDS-PAGE, transferred to nitrocellulose membranes and evaluated by chemiluminescent Western blot for pan-Cadherin, COX-IV and Hsp90. (Tissue blots were imaged using the MyECL Imager; cultured cell blots were imaged by exposure to X-ray film.)

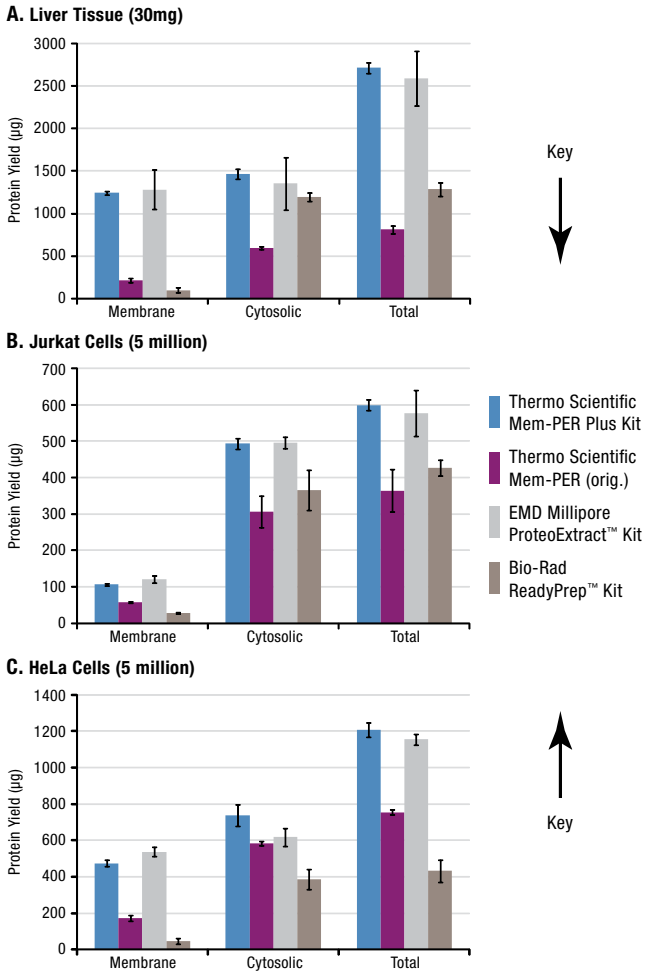

Figure 3. Improved protein yield using the Thermo Scientific Mem-PER Plus Membrane Protein Extraction Kit. Membrane proteins were isolated from mouse liver tissue, Jurkat cells and HeLa cells using four commercial extraction kits. Protein yields (micrograms) for membrane, cytosolic and total fractions were determined with the Pierce BCA Protein Assay Kit (Product # 23225).

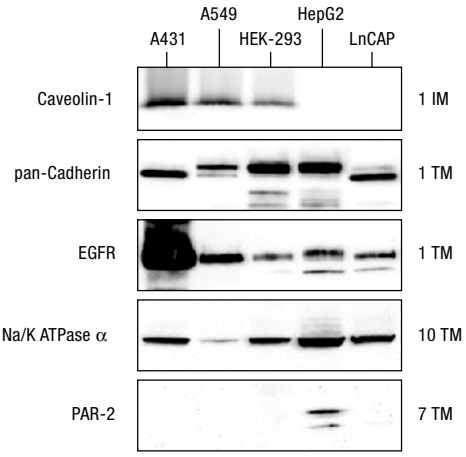

Figure 4. Efficient extraction of multiple membrane spanning proteins in various cell lines. Membrane proteins were isolated from 5 x 10^6 cultured cells following the Mem-PER Plus Membrane Protein Extraction Kit protocol. Membrane fractions (30mg) were separated by SDS-PAGE, transferred to nitrocellulose membranes and probed with antibodies against the corresponding protein and HRP-tagged secondary antibody. Blots were developed with SuperSignal West Dura Substrate and 1-minute exposures in the myECL Imager.

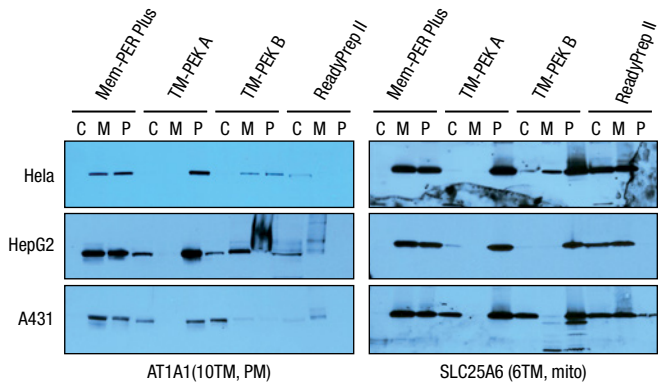

Figure 5. Higher extraction efficiency of multi-spanning integral membrane protein using sequential detergent extraction method. Integral membrane proteins sodium potassium ATPase alpha 1 (AT1A1) and ADP/ATP Translocase 3 (SLC25A6) were enriched using sequential detergent extraction method (Mem-PER Plus Kit) and compared to non-detergent-based methods (TM-PEK A and B) and sodium carbonate methods (ReadyPrep II).

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Ordering Information

Product #	Description	Pkg. Size
89842	**Mem-PER Plus Membrane Protein Extraction Kit** *Sufficient for 50 samples containing 5 million cells each or 25 samples of 20 to 40mg tissue each.*	Kit
	Contents: Cell Wash Solution	225mL
	Permeabilization Buffer	50mL
	Solubilization Buffer	25mL

Thermo Scientific Pierce Cell Surface Protein Isolation Kit

Purify cell surface proteins using selective biotinylation.

The Thermo Scientific™ Pierce™ Cell Surface Protein Isolation Kit is a complete set of reagents for selective biotinylation and subsequent purification of mammalian cell surface proteins to the exclusion of intracellular proteins.

The kit efficiently labels proteins that have accessible lysine residues and sufficient extracellular exposure. The isolation procedure uses a cell-impermeable, cleavable biotinylation reagent (Sulfo-NHS-SS-Biotin) to label exposed primary amines of proteins on the surface of intact adherent or suspension cells. Treated cells are then harvested, lysed and the labeled surface proteins are affinity-purified using Thermo Scientific™ NeutrAvidin™ Agarose Resin. The isolated cell surface proteins contain a small, nonreactive tag of the originally labeled primary amines, but are no longer biotinylated (biotin remains bound to the resin). The kit contains sufficient reagents for eight experiments, each involving four T75 flasks of confluent cells.

Highlights:

- **Isolates cell surface proteins** – reduces complexity of total cellular protein
- **Efficiently recovers labeled proteins** – cleavable biotin allows for nearly 100% recovery of isolated cell surface proteins
- **Convenience** – includes all reagents and complete instructions for labeling, cell lysis and purification of cell surface membrane proteins
- **Western blotting applications** – proteins recovered in SDS-PAGE buffer are loaded directly onto polyacrylamide gels
- **Robust system** – protocol designed for diverse cell lines, including NIH 3T3, HeLa, C6 and A431
- **Validated in** – heart and liver tissue and HeLa, NIH 3T3, A431 and C6 cultured mammalian cells

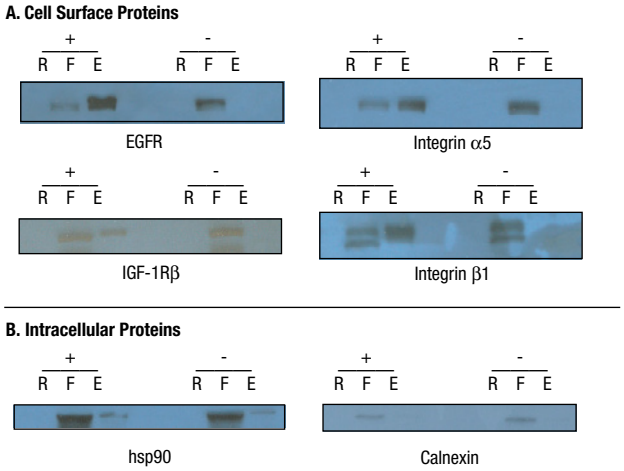

Figure 2. Specific extraction and isolation of cell surface proteins. HeLa cells were treated with or without Thermo Scientific™ EZ-Link™ Sulfo-NHS-SS-Biotin and processed with the Pierce Cell Surface Protein Isolation Kit protocol. Elution fractions, post-elution resin and flow-through were analyzed by Western blot for **A.** cell surface proteins EGFR, IGF-1Rβ, integrin β1 and integrin α5, and **B.** intracellular proteins, including heat shock protein 90 (hsp90) and calnexin. Legend: (+) label, (-) no label, (F) flow-through, (R) NeutrAvidin Gel and (E) elution. Only labeled cell surface proteins are present in the elution fractions.

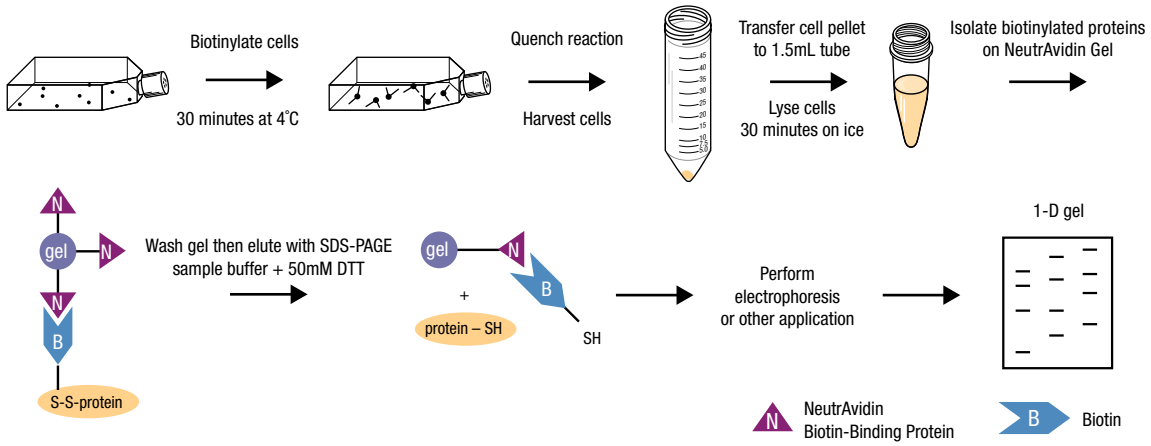

Figure 1. Protocol summary for the Thermo Scientific Pierce Cell Surface Protein Isolation Kit.

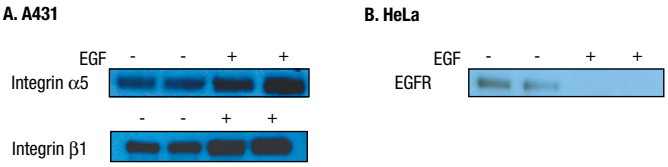

Figure 3. Differential expression of cell surface proteins in response to EGF. A431 and HeLa cells were treated with or without 20ng/mL and 10ng/mL EGF for 16 hours, respectively. Both cell types were processed with the Pierce Cell Surface Protein Isolation Kit protocol. Elution fractions were analyzed by Western blot for the quantities of **A**. integrin β1 and integrin α5 subunits or **B.** EGFR.

Adherent or suspended cells are first incubated with Sulfo-NHS-SS-Biotin, a cleavable reagent. The cells are subsequently lysed with a mild detergent and then labeled proteins are isolated with a NeutrAvidin Agarose resin. The bound proteins are recovered by incubating the resin with SDS-PAGE sample buffer containing 50mM DTT. The reducing agent cleaves the disulfide bond within the spacer arm of the biotinylation reagent (Figure 1). Nearly 100% of the bound proteins are released (Figure 2). The protocol is optimized for diverse cell lines including NIH 3T3, HeLa, C6 and A431. Isolated proteins can be analyzed by Western blot, allowing for differential expression analysis between treated and untreated cells (Figure 3), or between two or more cell lines.

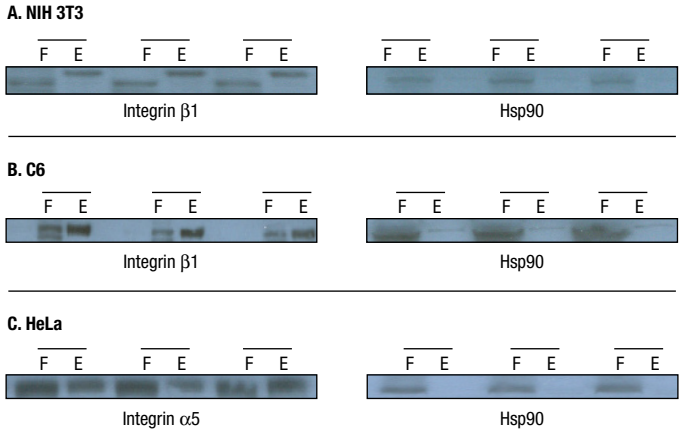

Figure 4. Validation of protocol with multiple cell lines. NIH 3T3, C6 and HeLa cells were labeled with Sulfo-NHS-SS-Biotin and triplicate samples were processed as described. Flow-through and elution fractions were analyzed by Western blot for cell surface proteins or Hsp90, an intracellular protein. **A.** NIH 3T3, **B.** C6 and **C.** HeLa.
Legend: **(F)** flow-through and **(E)** elution.

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Ordering Information

Product #	Description	Pkg. Size
89881	**Pierce Cell Surface Protein Isolation Kit** *Sufficient for eight experiments, each involving four T75 flasks of confluent cells.*	Kit
	Includes: EZ-Link Sulfo-NHS-SS-Biotin	8 x 12mg
	Quenching Solution	16mL
	Lysis Buffer	4.5mL
	NeutrAvidin Agarose	2.25mL
	Wash Buffer	34mL
	Dithiothreitol (DTT)	8 x 7.7mg
	PBS Packs (makes 500mL)	2 packs
	TBS Pack (makes 500mL)	1 pack
	Spin Columns	10 columns
	Collection Tubes	20 tubes

Subcellular Protein Fractionation and Organelle Isolation from Tissues and Cultured Mammalian Cells

Thermo Scientific Subcellular Protein Fractionation Kits for Cultured Cells or Tissues

Segregate and enrich proteins from five cellular compartments.

The Thermo Scientific™ Subcellular Protein Fractionation Kits enable segregation and enrichment of proteins from five different cellular compartments. Both kits include a combination of reagents for stepwise separation and extraction of cytoplasmic, membrane, nuclear soluble, chromatin-bound and cytoskeletal proteins from mammalian cells obtained from culture or isolated from tissue. However, the Thermo Scientific™ Subcellular Protein Fractionation Kit for Tissue was specifically developed to address the unique structures present in many different tissue types, such as heart, kidney, brain, liver, spleen and lung.

Extracts obtained with either kit generally have less than 15% contamination between fractions, which is sufficient purity for most protein localization and redistribution experiments. The extracts are compatible with a variety of downstream applications, including Western blotting; protein assays, including the Pierce BCA Protein Assay (Product # 23225); electrophoretic mobility shift assays, including the Thermo Scientific™ LightShift™ Chemiluminescent EMSA Kit (Product # 20148); and reporter gene and enzyme activity assays.

Highlights:

- **Efficient and complete** – extract functional cytoplasmic, membrane, nuclear soluble, chromatin-bound and cytoskeletal protein fractions with less than 15% cross contamination in < 3 hours from a single sample
- **Optimized** – formulations and protocols specific for fractionation of either cells or tissue
- **Convenient** – perform a simple procedure without using gradient ultracentrifugation
- **Sample specific** – separate kits developed and optimized for use with cultured mammalian cells or tissues
- **Compatible** – use extracts for downstream applications such as protein assays, Western blotting, gel-shift assays and enzyme activity assays
- **Thermo Scientific™ Pierce™ Tissue Strainer** – tissue kit includes convenient device to quickly remove tissue debris from homogenate using gravity filtration or centrifugation
- **Robust** – validated in brain, heart, liver, kidney, lung and spleen tissue and in Hela, NIH 3T3, HEK293 and A549 cultured mammalian cells

Applications:

- Determine a protein's cellular location
- Extract and enrich proteins from different cellular compartments
- Study protein translocation

The Thermo Scientific™ Subcellular Protein Fractionation Kits for Cultured Cells or Tissues contain four extraction buffers, a stabilized nuclease and Halt Protease Inhibitor Cocktail. The Thermo Scientific™ Subcellular Protein Fraction Kit for Cultured Cells contains reagents sufficient to fractionate 50 cell pellets, each containing 2 million cells (20µL packed). The kit for tissue samples can process up to 25 samples of up to 200mg each. The sample handling for each kit is similar, the exception being that the kit for tissues requires mechanical homogenization in the cytoplasmic extraction buffer (CEB) to disrupt the tissues into a single-cell suspension, while the kit for cultured cells starts with a pellet of individual, cultured cells. The first reagent (CEB) in both kits causes selective permeabilization of the cell membrane, releasing soluble cytoplasmic contents. The second reagent in both kits dissolves plasma, mitochondria and ER-golgi membranes but does not solubilize the nuclear membranes. After recovering intact nuclei by centrifugation, a third reagent yields the soluble nuclear extract. An additional nuclear extraction with micrococcal nuclease is performed to release chromatin-bound nuclear proteins. The recovered insoluble pellet is then extracted with the final reagent to isolate cytoskeletal proteins (Figure 1).

As with the NE-PER Nuclear and Cytoplasmic Extraction Kit, soluble nuclear extracts generated using the Subcellular Fractionation Protein Kit are compatible with gel-shift assays to further characterize transcription factor activation states (Figure 4).

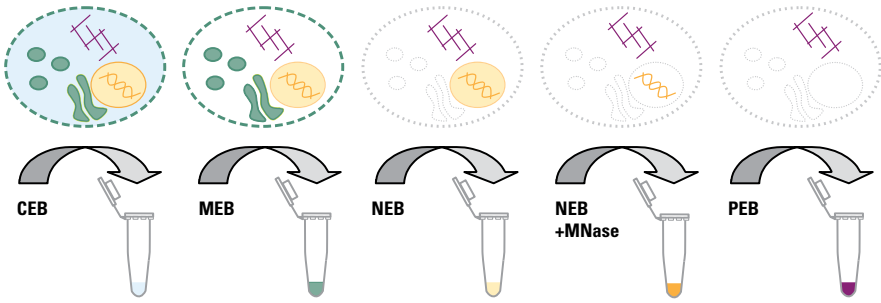


Figure 1. Schematic of the subcellular fractionation procedure. Cellular compartments are sequentially extracted by incubating cells with cytoplasmic extraction buffer (CEB) followed by membrane extraction buffer (MEB) and nuclear extraction buffer (NEB). Adding micrococcal nuclease to NEB extracts chromatin-bound proteins from the cell pellet before adding the pellet extraction buffer (PEB) to solubilize cytoskeletal proteins.

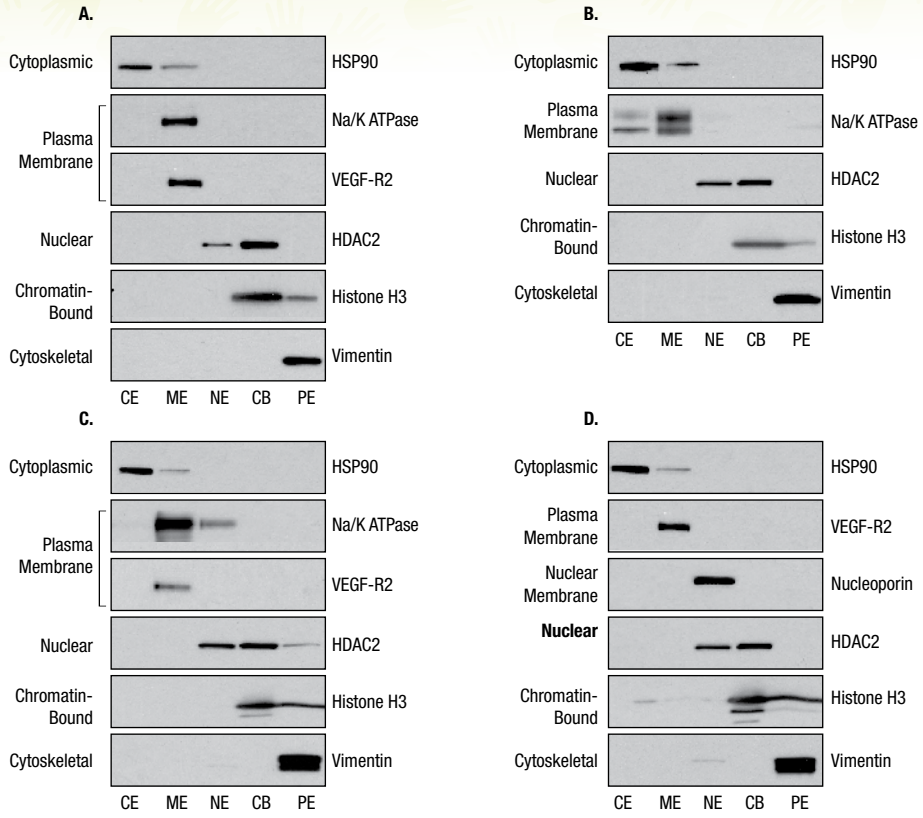


Figure 2. Fractions from multiple tissue samples resulted in minimal cross-contamination of target proteins. Mouse tissue samples were fractionated using the Subcellular Protein Fractionation Kit for Tissues. Normalized loads of each extract (10µg) were analyzed by Western blotting. (Data not shown for liver and spleen.) **CE:** cytoplasmic extract; **ME:** membrane extract; **NE:** nuclear extract; **CB:** chromatin-bound extract; **PE:** pellet extract.

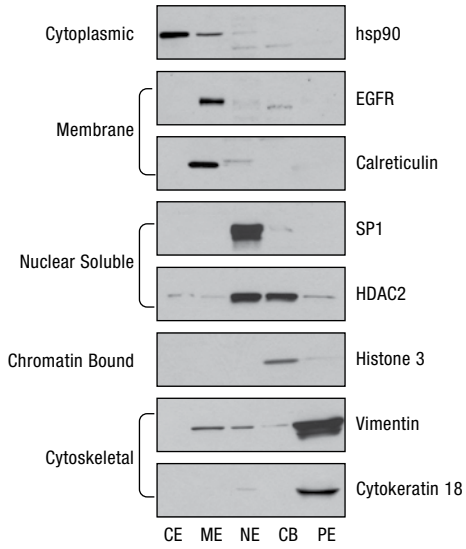


Figure 3. Western blots of fractionated cellular proteins. HeLa cells (2 x 10^6) were fractionated using the Subcellular Protein Fractionation Kit. Normalized portions of each extract (10µg) were analyzed by Western blotting using specific antibodies against proteins from various cellular compartments, including cytoplasmic (HSP90), plasma membrane (EGFR), endoplasmic reticulum (calreticulin), nuclear soluble (SP1 and HDAC2), chromatin-bound (histone 3) and cytoskeleton (cytokeratin 18 and vimentin). The blots were probed with goat anti-rabbit (H+L) HRP or goat anti-mouse (H+L) HRP and detected with SuperSignal West Dura Chemiluminescent Substrate (Part # 34076). The results demonstrate clear fractionation of each protein with low cross contamination among fractions.

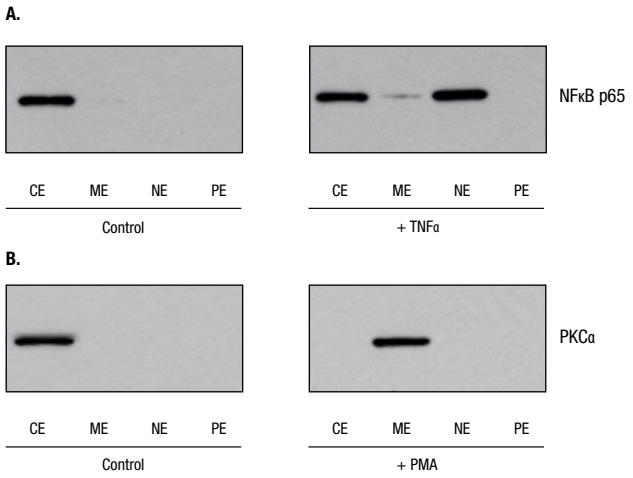
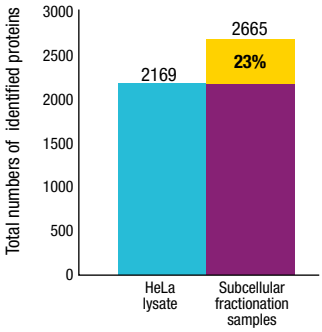


Figure 4. Western blot analysis of protein translocation. Top Panel: A549 cells (2 x 10^6) were either mock-treated (-) or incubated (+) with 20µg/mL TNFα for 20 minutes and fractionated using the Subcellular Protein Fractionation Kit. Each extract (10µg) was analyzed by Western blot using an anti-NFκB p65 antibody. **Bottom Panel:** Serum-starved HeLa cells (2 x 10^6) were either mock-treated (-) or incubated (+) with 1µM PMA for 20 minutes and fractionated. Each extract (10µg) was analyzed by Western blot using an anti-PKCα antibody. Goat anti-rabbit or anti-mouse (H+L) HRP was used as the secondary antibody and SuperSignal West Dura Chemiluminescent Substrate (Product # 34076) was used for detection.

Subcellular Protein Fractionation and Organelle Isolation from Tissues and Cultured Mammalian Cells



Subcellular Fractions	Number of Unique Proteins
CE	232
ME	336
NE	371
CB	201
PE	137

23% Increase in Protein Identification in Subcellular Fractionation Samples

Figure 5. Subcellular fractionation enhances MS proteome coverage. In a comparison of total numbers of proteins identified in HeLa whole lysate and subcellular fractionation via LC-MS/MS, data showed that 2665 proteins were identified in subcellular fractionation samples and 2169 proteins identified in HeLa whole cell lysate. Subcellular fractionation provided an increase of approximately 23% proteome coverage compare to whole cell lysate. Table shows total numbers of those unique proteins identified in each fraction but not found in whole cell lysate.

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Ordering Information

Product #	Description	Pkg. Size
78840	**Subcellular Protein Fractionation Kit for Cultured Cells** *Sufficient for 50 samples each containing 2 million cells (20µL packed).*	Kit
	Includes: Cytoplasmic Extraction Buffer (CEB)	10mL
	Membrane Extraction Buffer (MEB)	10mL
	Nuclear Extraction Buffer (NEB)	10mL
	Pellet Extraction Buffer (PEB)	5mL
	Micrococcal Nuclease	150µL
	Calcium Chloride, 100 mM	250µL
	Halt Protease Inhibitor Cocktail, 100X	350µL
87790	**Subcellular Protein Fractionation Kit for Tissue** *Sufficient for 25 extractions of 200mg tissue each.*	Kit
	Includes: Cytoplasmic Extraction Buffer (CEB) for Tissues	50mL
	Membrane Extraction Buffer (MEB) for Tissues	35mL
	Nuclear Extraction Buffer (NEB) for Tissues	20mL
	Pellet Extraction Buffer (PEB) for Tissues	6.5mL
	Calcium Chloride, 100mM	450µL
	Micrococcal Nuclease, 100 units/µL	260µL
	Halt Protease Inhibitor Cocktail (100X)	1mL
	Pierce Tissue Strainers, 250µm	25 each
88216	**Micrococcal Nuclease**	150µL

Thermo Scientific Pierce Tissue Strainer

The Thermo Scientific™ Pierce™ Tissue Strainer can be used to quickly separate cells or cell lysates from tissue debris following mechanical disruption. The debris is separated by passing homogenates through a 250µm mesh filter using gravity or gentle centrifugation. The easy-to-use Pierce Tissue Strainers are made of strong nylon and fit into standard 15mL conical tubes, making the processing and recovery of samples simple and reliable. The strainers can be filled with 2.5mL of cells or lysate at one time, although multiple loads are possible with stepwise sample application.

The Pierce Tissue Strainers can be used with the Subcellular Protein Fractionation Kit for Tissue and other protein extraction reagent protocols that utilize tissue samples. Removal of large tissue debris facilitates easier sample handling and can protect downstream instrumentation and devices by eliminating fouling of injection ports and columns.

Highlights:

- **Convenient** – remove debris from tissue homogenate within minutes using gravity filtration or centrifugation
- **Compatible** – unique design fits into standard 15mL conical tubes
- **Versatile** – remove debris from a variety of tissues types, including liver, heart, brain, kidney, lung and spleen

Ordering Information

Product #	Description	Pkg. Size
87791	**Pierce Tissue Strainers, 250µm, 2.5mL** *Sufficient for 50 separations, 2.5mL per load of tissue homogenate.*	50 units

Thermo Scientific Mitochondria Isolation Kits for Cultured Cells or Tissue

Isolate intact mitochondria with maximum yield.

The Thermo Scientific™ Mitochondria Isolation Kits for Cultured Cells or Tissue provide a versatile, reagent-based method for isolating intact mitochondria from either cultured mammalian cell samples or tissue samples.

The isolation of mitochondria is typically a laborious process requiring single-sample processing with Dounce homogenization. The Mitochondria Isolation Kits use a non-mechanical, reagent-based method (Figure 1A) that allows multiple samples to be processed concurrently and results in maximum yield of mitochondria with minimal damage to integrity. The kits also offer a second isolation method based on traditional Dounce homogenization (Figure 1B), which results in 2-fold more mitochondria recovery, as determined by protein assay. Both methods use differential centrifugation to separate the mitochondrial and cytosolic fractions with a bench-top microcentrifuge and are completed in approximately 40 minutes (post-cell harvest) for cultured mammalian cells, or 60 minutes for soft or hard tissue samples. Once isolated, the mitochondria can be used in downstream applications such as apoptosis, signal transduction and metabolic studies, as well as to facilitate mitochondrial proteomics efforts.

Highlights:

- **Fast** – isolate intact mitochondria in approximately 40 minutes
- **Multi-sample format** – reagent-based method allows for concurrent preparation of multiple samples
- **Optional alternate method** – reagents and protocol included for traditional Dounce homogenization
- **Benchtop-compatibility** – isolation performed in a microcentrifuge tube
- **Validated in** – heart and liver tissue and HeLa, A431, A549, C6, Cos7 and Hepa cultured mammalian cells

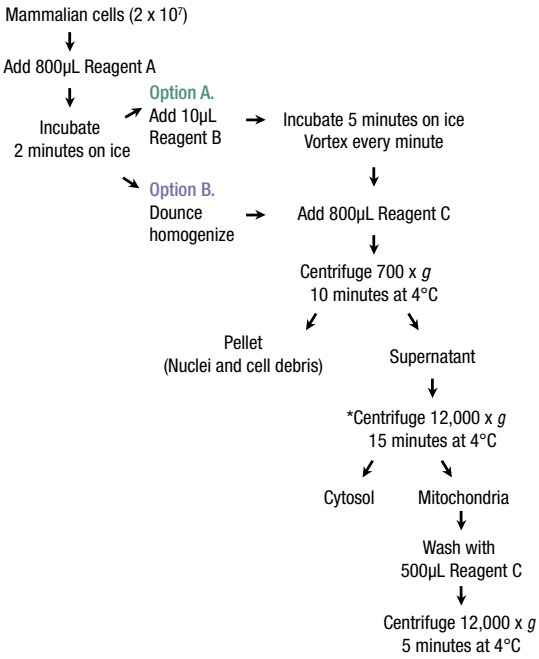

Figure 1. Procedure for the isolation of mitochondria from cultured mammalian cells using the reagent-based method (A) and the Dounce-based method (B).

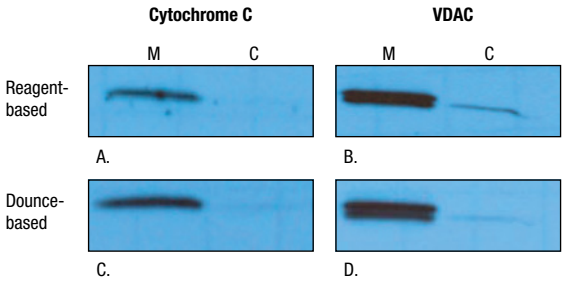

Figure 2. Analysis of mitochondrial integrity following isolation from cultured cells. Mitochondrial and cytosol fractions were prepared from C6 cells using the reagent-based method **(A and B)** or Dounce homogenization **(C and D).** Fractions were analyzed via Western blot for cytochrome C **(A and C)** or voltage-dependent anion channel (VDAC) **(B and D)**. SuperSignal West Pico Chemiluminescent Substrate (Product # 34080) was used for detection. **M** = mitochondrial and **C** = cytosol.

Figure 3. Analysis of cytosolic contamination in mitochondrial fraction. Mitochondrial and cytosol fractions were prepared from NIH 3T3 cells. Each fraction was analyzed by Western blot for the cytosolic protein, hsp90. **M** = mitochondrial, and **C** = cytosol.

Subcellular Protein Fractionation and Organelle Isolation from Tissues and Cultured Mammalian Cells

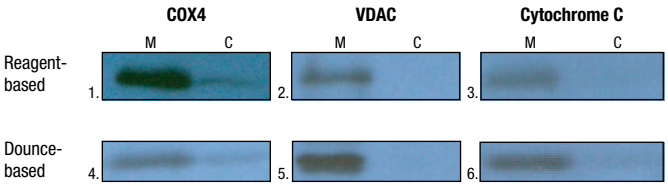

Figure 4. Analysis of mitochondrial integrity following isolation from tissue. Mitochondrial **(M)** and cytosolic **(C)** fractions were prepared from fresh rat liver **(Panels 1, 2, 3, 5 and 6)** and heart **(Panel 4)** tissue samples using the reagent-based and Dounce homogenization methods. Fractions were analyzed via Western blot for COX4, voltage-dependent anion channel (VDAC) and cytochrome C. SuperSignal West Pico Chemiluminescent Substrate (Product # 34080) was used for detection. COX4 is an inner-mitochondria membrane protein, VDAC is an outer-mitochondria membrane protein and cytochrome C is located in the intermembrane space.

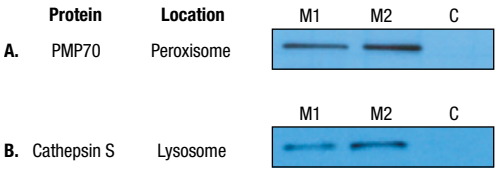

Figure 5. Reduction of lysosomal and peroxisomal contaminants in mitochondrial fraction. Mitochondrial and cytosol fractions were prepared using a modified reagent-based isolation method. Heavy, more purified mitochondria were collected at 3,000 x *g* and the supernatant was centrifuged at 12,000 x *g* to collect remaining mitochondria. Each fraction was analyzed by Western blot for **A.** Peroxisomal membrane protein 70 (PMP70, C6 cells) and **B.** Lysosomal Cathepsin S (NIH 3T3 cells). **M1** = 3,000 x *g* mitochondrial fraction, **M2** = 12,000 x *g* mitochondrial fraction and **C** = cytosol.

***See Table 1 below for protein quantification.*

Table 1. Collection of mitochondria (reagent-based method).

RCF *(x g)*	Protein (µg)	% Total Protein
3,000	159.1	62
12,000	98.2	38

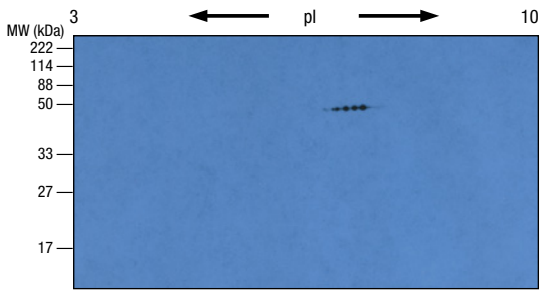

Figure 6. 2-D Western blot of superoxide dismutase (Mn-SOD) in isolated mitochondria. Mitochondria were isolated from NIH 3T3 cells using the Dounce method, resolved by 2-D gel electrophoresis and analyzed by Western blot for manganese-containing SOD. Approximately 15µg of mitochondrial protein was focused on an 11cm, pH 3-10 IPG strip. The second dimension was performed using 8-16% SDS-PAGE. *A more purified preparation of mitochondria can be obtained by centrifuging at 3,000 x g instead of 12,000 x g.*

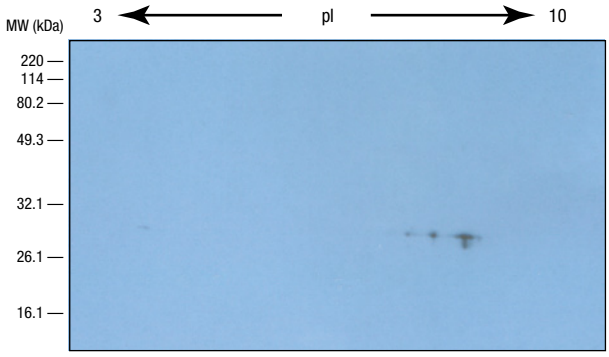

Figure 7. 2-D Western blot of superoxide dismutase (Mn-SOD) in isolated mitochondria. Intact mitochondria from the liver of a female Sprague-Dawley rat was processed using the Dounce homogenization method. The isolated mitochondria were lysed using M-PER Mammalian Protein Extraction Reagent (Product # 78501) and approximately 35µg of total mitochondrial protein was added to 2-D sample buffer (8M urea, 4% CHAPS, pH 5-8 carrier ampholytes, 50mM DTT). Proteins were resolved on a pH 5-8 IPG strip followed by 8-16% SDS-PAGE and analyzed by Western blot for Mn-SOD.

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Ordering Information

Product #	Description	Pkg. Size
89874	**Mitochondria Isolation Kit for Cultured Cells** *Sufficient for 50 applications.* Includes: Mitochondria Isolation Kit Reagent A Mitochondria Isolation Kit Reagent B Mitochondria Isolation Kit Reagent C	Kit 50mL 500µL 70mL
89801	**Mitochondria Isolation Kit for Tissue** *Sufficient for 50 isolations of intact mitochondria from soft and hard tissue.* Includes: Mitochondria Isolation Kit Reagent A Mitochondria Isolation Kit Reagent B Mitochondria Isolation Kit Reagent C BSA BupH Phosphate Buffered Saline	Kit 50mL 500µL 65mL 235mg 1 pack

Thermo Scientific Lysosome Enrichment Kit for Tissues and Cells

Enables efficient subcellular fractionation.

The Thermo Scientific™ Lysosome Enrichment Kit has been optimized for the subcellular fractionation and isolation of intact lysosomes by high-speed density gradient centrifugation for proteomics analysis.

This kit enables the enrichment of intact lysosomes from cells and tissue. The kit uses density gradient centrifugation to separate lysosomes from contaminating cellular structures. The isolated lysosomes may be used for a number of downstream applications, including 2-D/MS, electron microscopy, disease profiling, gene expression, signal transduction, and interaction or localization studies.

Highlights:

- **Efficient and easy to use** – kit reagents and gradient centrifugation separate lysosomes from contaminating structures (Table 1)
- **Compatible** – prepare samples for downstream applications, including 2-D/MS, electron microscopy, disease profiling, gene expression, signal transduction, and interaction or localization studies
- **Validated in** – heart and liver tissue and HeLa, A431, A549, C6, Cos7 and Hepa cultured mammalian cells

Table 1. The Thermo Scientific Lysosome Enrichment Kit is a convenient and fast means for sample preparation.

Target Organelle	Sample Source	OptiPrep™ Density Gradient	Centrifugation Speed (× *g*)	Centrifugation Time (Minutes)
Lysosome	Cells Tissue (soft & hard)	15%, 17%, 20%, 23%, 27% and 30%	145,000	120

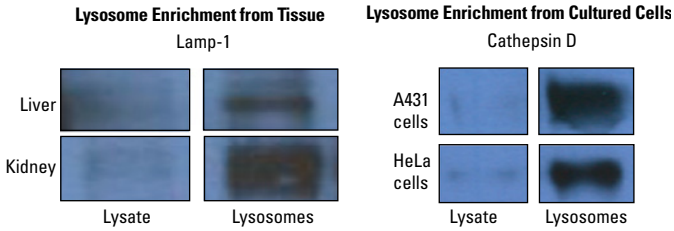

Figure 1. Lysosome enrichment from tissue and cultured cells. Left: Liver and kidney tissues (200mg each) were processed and isolated lysosomes were analyzed by Western blotting for Lamp-1, a lysosomal membrane protein marker. **Right:** Total cell lysate and isolated lysosomes were analyzed by Western blotting for Cathepsin D, a membrane-bound and soluble lysosome marker. Both samples were processed using the Lysosome Enrichment Kit for Tissue and Cultured Cells.

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Ordering Information

Product #	Description	Pkg. Size
89839	**Lysosome Enrichment Kit for Tissues and Cultured Cells** *Sufficient for 25 applications.*	Kit
	Includes: Lysosome Enrichment Reagent A	90mL
	Lysosome Enrichment Reagent B	90mL
	OptiPrep Cell Separation Media	50mL
	BupH Phosphate Buffered Saline	1 pack
Complementary Organelle Antibodies		
89917	**Lamp-1 Monoclonal Antibody**	50µg

introduction

In addition to mammalian systems, other cells or tissues are utilized in life science research as model organisms. The methods used for lysis depend on the sample type and the downstream application.

Non-mammalian sample types

Model organisms are used to understand specific biological systems, based on their similarities with the pathways of more complex organisms, but with the advantage of being studied more easily, rapidly and inexpensively than their more complex counterpart. Bacteria, specifically *E. coli*, were among the first organisms studied for a better understanding of molecular genetics. Simple eukaryotic organisms, such as yeast, have also been widely used in genetics and cell biology, and are also easy to culture and manipulate, with a cell cycle and regulatory proteins that are very similar to the more complex mammalian systems.

Not only are bacteria and yeast useful as model organisms, but they can also serve as protein expression systems. *E. coli* has been the most popular means of producing recombinant proteins due to its low cost, ease of use, and long history of producing a wide variety of proteins. However, expression in bacterial systems is limited because bacteria do not contain the appropriate enzymatic systems for eukaryotic post-translational modifications (PTMs) or proper folding and may produce nonfunctional proteins or inclusion bodies. Yeast expression systems allow for the stable production of proteins that are similar to those expressed in mammalian cells at a fairly high yield, but cannot produce all mammalian PTMs. *Baculovirus*-infected insect cells grown in suspension or monolayer culture enable the expression of glycosylated proteins that cannot be produced using yeast or bacterial cell in higher quantities than in mammalian systems.

In agricultural biotechnology, plant genomes are being studied and manipulated in order to improve crop yield, increase resistance to diseases and adverse weather, and to enhance the nutritional content of foods. Efficient isolation of active proteins from different plant species is critical for determining the effectiveness of genetic modifications.

Thermo Scientific Protein Extraction Products for Non-mammalian Tissues and Cells

For these non-mammalian cells and tissues, lysis has traditionally required harsh mechanical disruption, such as sonication or French press (bacteria), glass bead beating (yeast), or liquid nitrogen/freeze-grinding (plants). We offer optimized lysis products for the rapid extraction of total protein from these cells and tissues without the need for this type of equipment. All reagents and kits use gentle non-denaturing detergents to prepare cell lysates that are compatible with typical downstream assays, such as SDS-PAGE, Western blotting and immunoprecipitation. Protease and/or phosphatase inhibitor cocktails are recommended during lysis to protect protein structure and phosphorylation states.

Table 1. Overview of non-mammalian sample types and recommended Thermo Scientific Protein Extraction Reagents or Kits.

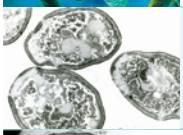
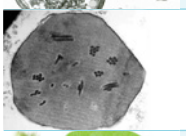
	Sample Type	Goal	Recommended Thermo Scientific Reagents or Kits	Product Information and Data
	Bacterial Cells	Total Protein Extraction	B-PER Reagent	p. 36
	Yeast Cells	Total Protein Extraction	Y-PER Reagent	p. 39
	Insect Cells (*Baculovirus*)	Total Protein Extraction	I-PER Reagent	p. 41
	Plant Tissue (Leaf, Stem, Roots, Flowers)	Total Protein Extraction	P-PER Reagent	p. 42

Table 2. Product compatibility guide for protein extraction from non-mammalian cells and tissues.

			PROTEIN ASSAYS				DOWNSTREAM COMPATIBILITY			
Kit	**Product #**	**Validated in**	**BCA**	**Commassie Plus**	**660nm**	**Detergent Compatible Bradford**	**SDS-PAGE, Western Blotting**	**Protein Purification**	**Reporter Assays**	**Other**
B-PER and B-PER (2X) Reagents	**78243 78248 90084 78250 78266**	*E. coli, B. subtilis*	Yes	Yes	Yes, dilute 1:2	Yes	Yes	Yes		
B-PER Complete Reagent	**89821 89822**	*E. coli, B. subtilis*	Yes		Yes	Yes, subtract background from enzymes	Yes	Yes		
Y-PER and Y-PER Plus Reagents	**78991 78990 78998 78999**	*B. subtilis, S. cerevisiae, S. pombe and P. pastoris*	Yes			Yes	Yes	Yes	Yes	Genomic and Plasmid DNA Extraction
I-PER Reagent	**89802**	sf9	Yes			Yes	Yes	Yes		
P-PER Reagent	**89803**	Leaf, Root, Stem and Flower Tissue in *Arabidopsis*, Tobacco, Maize, Soybean, Pea and Spinach Plants	Yes, RAC-BCA				Yes	Yes		Activity Assays

▸ Learn more at thermofisher.com/proteinextraction

Thermo Scientific B-PER Bacterial Protein Extraction Reagents

Simple, convenient protein extraction from bacteria.

Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagents are designed to extract soluble protein from bacterial cells without harsh chemicals or mechanical disruption. These easy-to-use cell lysis reagents are nonionic detergent-based solutions that effectively disrupt cells and solubilize native or recombinant proteins without denaturation.

Highlights:

- **Ready to use** – one-step cell lysis of gram-positive and gram-negative bacteria using a mild, nonionic detergent (proprietary) in Tris or phosphate buffer formulations
- **Fast and simple** – just add B-PER Reagent to a bacterial pellet, incubate with mixing for 10 to 15 minutes and recover soluble proteins after pelleting the cell debris
- **Convenient** – Thermo Scientific™ B-PER™ Complete Reagent contains lysozyme and a universal nuclease in a single formulation with 4°C storage
- **Excellent yields** – recover recombinant proteins from bacterial lysates or purify inclusion bodies to near-homogeneous levels
- **Flexible** – B-PER Reagents are suitable for any scale of protein extraction and are available in phosphate and 1X and 2X Tris formulations, with or without enzymes
- **Compatible** – completely compatible with addition of protease inhibitors; the resulting protein extract can be used in protein assays, typical affinity purification methods (e.g., GST, 6xHis) and other applications

B-PER Bacterial Extraction Reagents are more effective than traditional sonication and typical homemade lysis buffers, many of which include detergents and components that interfere with downstream applications. B-PER Reagents are formulated in Tris or phosphate buffer at physiological pH. They extract native and soluble recombinant proteins and yield lysates that are directly compatible with most downstream workflows such as electrophoresis, affinity purification, immunoprecipitation, protein interaction analysis, crosslinking and protein labeling.

B-PER Complete Reagent contains optimized concentrations of both lysozyme and Thermo Scientific™ Pierce™ Universal Nuclease. Lysozyme facilitates lysis by solubilizing bacterial cell walls. Pierce Universal Nuclease reduces the viscosity of bacterial extracts and improves downstream applications by digesting DNA and RNA. B-PER Complete Reagent is most efficient for frozen cells but has been validated and optimized to achieve high yields with both fresh and frozen cells from gram-positive and gram-negative bacteria. (For optimal performance with fresh gram-negative bacteria, supplementation with 1mM EDTA is required.) B-PER Complete Reagent is compatible with GST-fusion protein purification, unlike other formulations of lysis buffers that may inhibit enzyme function.

Convenient, Ready-to-use Formats

Table 1. Thermo Scientific B-PER Reagent Selection Guide.

B-PER Product	Composition and Suitable Applications
B-PER Complete Reagent	• Detergent in Tris buffer with lysozyme and Pierce Universal Nuclease • Improved cell lysis and DNA digestion, thereby releasing soluble proteins and reducing viscosity to increase yields • Recovery of high-molecular weight proteins • Cost-effective equivalent to Bugbuster™ Master Mix
B-PER Reagent	• Detergent in Tris buffer; no enzyme components • Bacterial lysis • Purification of affinity-tagged proteins
B-PER II (2 x B-PER) Reagent	• 2X B-PER (detergent in Tris buffer; no enzyme components) • Bacterial lysis for low cell density • Purification of proteins having low expression levels
B-PER Reagent (in Phosphate Buffer)	• Detergent in sodium phosphate buffer; no enzyme components • Amine-free formulation for direct compatibility of lysate with amine-reactive labeling and crosslinking

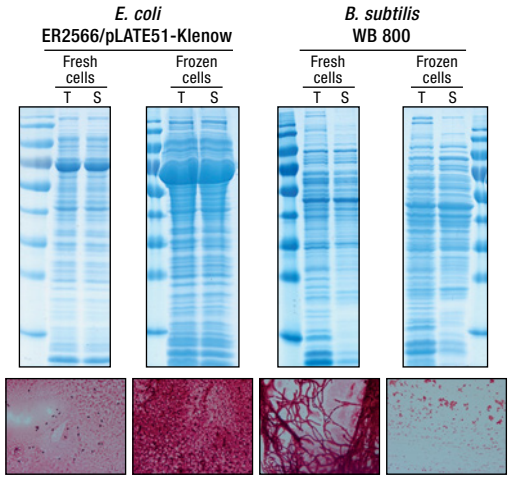


Figure 1. Effective cell lysis of Gram-negative and Gram-positive bacteria with Thermo Scientific B-PER Complete Reagent. Fresh or frozen cells of *E. coli* and *Bacillus* were lysed in reagent and then protein fractions were separated by SDS-PAGE (10% gels) alongside a protein ladder (Product # 26616). Total (T) protein fraction is the initial lysate before removal of cell debris by centrifugation; the soluble (S) protein fraction is the final clarified lysate. Micrographs below are the corresponding fuschin-stained samples of cells after lysis.

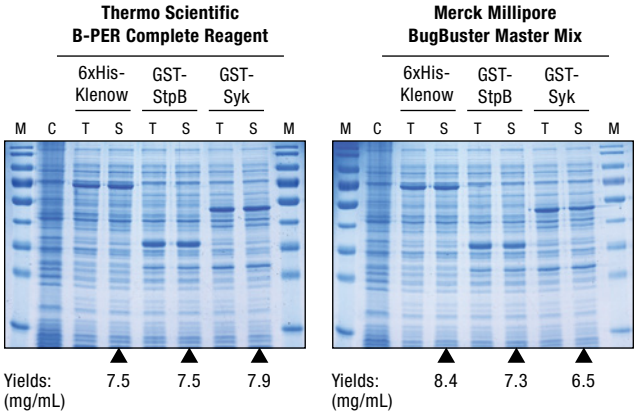


Figure 2. Protein yield comparison of two bacterial cell lysis reagents. *E. coli* ER2566/pLATE51-Klenow and ER2566/pGSH-Syk cell pellets (0.5g), were resuspended in 2.5mL aliquots of B-PER Complete Reagent or BugBuster Master Mix with gentle vortexing for 15 minutes at room temperature. Insoluble cell debris was removed by centrifugation at 16,000 x *g* for 20 minutes at 4°C. Protein yields (concentrations) for soluble fractions were determined using the Pierce BCA Protein Assay Kit (Product # 23225).

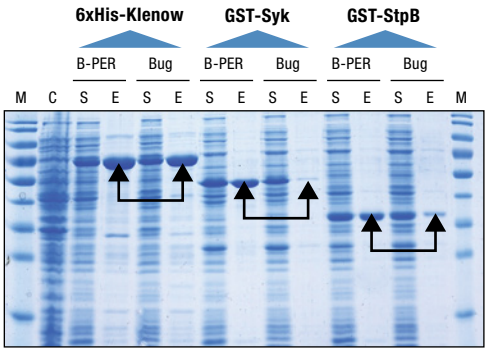


Figure 3. Thermo Scientific B-PER Complete Reagent is compatible with purification of 6xHis and GST fusion proteins. *E. coli* ER2566/pLATE51-Klenow and ER2566/pGSH-Syk cell pellets (0.5g) were resuspended in 2.5mL aliquots of B-PER Complete Reagent or BugBuster Master Mix with gentle vortexing for 15 minutes at room temperature. Insoluble cell debris was removed by centrifugation at 16,000 x *g* for 20 minutes at 4°C. His6-Klenow protein was purified using Thermo Scientific™ HisPur™ Ni-NTA Agarose (Product # 88221). GST-Syk protein was purified using Thermo Scientific™ Pierce™ Glutathione Agarose (Product # 16100). **L** = Thermo Scientific™ PageRuler™ Prestained Protein Ladder (Product # 26616); **C** = negative control (total proteins before induction IPTG); **S** = soluble proteins after induction with 0.1mM IPTG: **E** = elution fraction after protein purification.

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Ordering Information

Product #	Description	Pkg. Size
89821	**B-PER Complete Bacterial Protein Extraction Reagent** *Sufficient for lysis of 50g of bacterial biomass.*	250mL
89822	**B-PER Complete Bacterial Protein Extraction Reagent** *Sufficient for lysis of 100g of bacterial biomass.*	500mL
78243	**B-PER Bacterial Protein Extraction Reagent** *Sufficient for 40g cell paste.*	165mL
90084	**B-PER Bacterial Protein Extraction Reagent** *Sufficient for 60g cell paste.*	250mL
78248	**B-PER Bacterial Protein Extraction Reagent** *Sufficient for 125g cell paste.*	500mL
78266	**B-PER Reagent (in Phosphate Buffer)** *Sufficient for 125g cell paste.*	500mL
78260	**B-PER II Bacterial Protein Extraction Reagent (2X)** *Sufficient for 125g cell paste.*	250mL

Thermo Scientific Pierce Universal Nuclease for Cell Lysis

A highly active endonuclease that degrades all forms of DNA and RNA.

Thermo Scientific™ Pierce™ Universal Nuclease for Cell Lysis is ideal for a wide variety of applications where complete digestion of nucleic acids is needed when preparing cell lysates.

Pierce Universal Nuclease for Cell Lysis is a genetically engineered endonuclease from *Serratia marcescens*. The enzyme is produced and purified from *E. coli* and consists of two identical 30-kDa subunits with two critical disulfide bonds. This indiscriminate endonuclease degrades single-stranded, double-stranded, linear and circular DNA and RNA and is effective over a wide range of temperatures and pH. This enzyme has high specific activity (100-fold greater than DNase I) and increased thermal stability compared to other nucleases. Pierce Universal Nuclease is ≥ 99 pure enzyme, is free of any measurable protease activity and is supplied at 250U/µL. Pierce Universal Nuclease for Cell Lysis is identical in performance to Benzonase™ Nuclease (EMD Merck).

Highlights:

- **Broad spectrum** – degrades all forms of DNA and RNA
- **Highest-quality enzyme** – nuclease is ≥ 99% pure, as tested by SDS-PAGE
- **Robust activity** – 100-fold greater specific activity than DNase I
- **Versatile** – can be used with a wide variety of cell lysis reagents

Applications:

- Use with B-PER Reagent, Y-PER Reagent or other commercial or homebrew cell lysis reagents and/or mechanical disruption to reduce viscosity in protein extracts
- Remove DNA and RNA from recombinant protein preparations prior to downstream processing

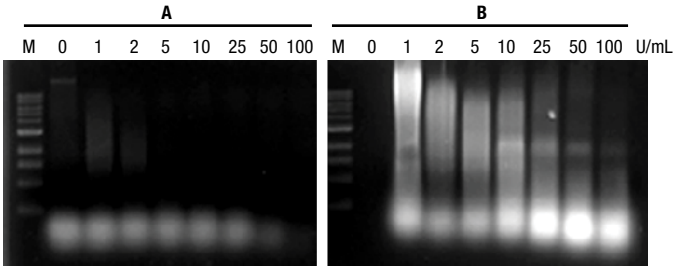

Figure 1. Thermo Scientific Pierce Universal Nuclease activity in cells lysed with Thermo Scientific B-PER Reagent. Cells were suspended in B-PER Reagent **(A)** or B-PER Reagent with lysozyme **(B)** with increasing concentrations of Pierce Universal Nuclease for Cell Lysis and incubated at room temperature for 30 minutes. The lysates were then cleared by centrifugation and resolved on a 1% agarose gel, and nucleic acids were stained with ethidium bromide and visualized under ultraviolet (UV) light. M = DNA ladder. Cells that were lysed with B-PER Reagent with lysozyme but without Pierce Universal Nuclease for Cell Lysis were too viscous to be loaded onto the gel. Both images are from the same gel but were separated for presentation.

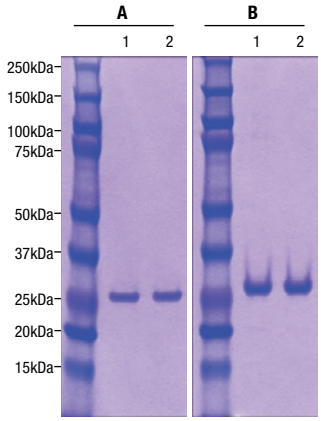

Figure 2. Thermo Scientific Pierce Universal Nuclease for Cell Lysis purity is comparable to that of other commercial nucleases. Pierce Universal Nuclease for Cell Lysis **(1)** and supplier E **(2)** were resolved by SDS-PAGE at a concentration of 3µg **(A)** or 8µg **(B)**. Band intensities were stained with Thermo Scientific™ Imperial Protein Stain (Product # 24615).

Ordering Information

Product #	Description	Pkg. Size
88700	Pierce Universal Nuclease for Cell Lysis *Sufficient for 200mL lysate.*	5kU
88701	Pierce Universal Nuclease for Cell Lysis *Sufficient for 1L lysate.*	25kU
88702	Pierce Universal Nuclease for Cell Lysis *Sufficient for 4L lysate.*	100kU
89833	Lysozyme	5g
90082	Lysozyme	0.5mL
89836	DNase I, RNase-free	1,000 units
90083	DNase I	0.5mL

Thermo Scientific Y-PER Yeast Protein Extraction Reagents

Extract proteins from yeast without glass beads or sonication.

The Thermo Scientific™ Y-PER™ Yeast Protein Extraction Reagents are the first commercially available yeast lysis reagents to use a mild detergent lysis procedure to rapidly and efficiently release functionally active solubilized proteins.

These detergent-based cell lysis buffers eliminate the need to use glass beads or mechanical disruption to break through the thick proteinaceous cell envelope to extract protein. Y-PER Reagents are effective for *S. cerevisiae* and other popular species, making them applicable for use in fusion-tagged protein purification and reporter enzyme assays with these model organisms. These lysis reagents also can be used for genomic and plasmid DNA extraction from yeast. In addition, Thermo Scientific™ Y-PER Plus Reagent is a Tris-based formulation that contains a fully dialyzable detergent and has very low ionic strength for downstream applications that are sensitive to these components.

Highlights:

- **Convenient –** ready-to-use room temperature reagent with a dialyzable detergent formulation option
- **Excellent yields** – extract more than twice as much protein as glass bead methods (Figure 1)
- **Easy to use** – eliminate the physical problems associated with traditional glass bead lysis (e.g., clinging static-charged beads, protein/bead clumps and runaway beads)
- **Optimized for yeast** – works with *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris* and *Bacillus subtilus*
- **Versatile** – effective for many different organisms, including *Bacillus subtilus* and both gram-positive and gram-negative bacteria; suitable for use in a diverse range of situations

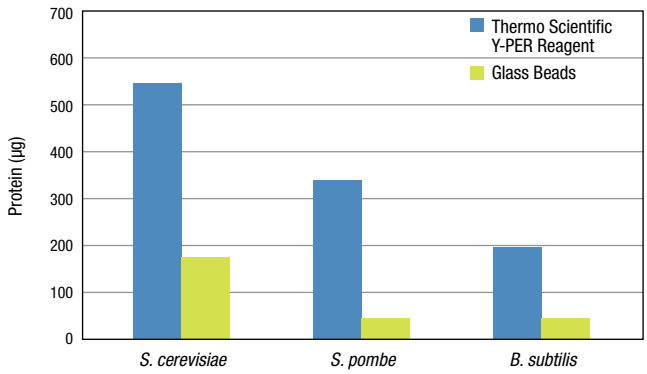

Figure 1. Thermo Scientific Y-PER Reagent extraction yields greater amounts of usable protein. In all three organisms tested, Y-PER Reagent extracts contain more usable protein than extracts from traditional glass bead lysis.

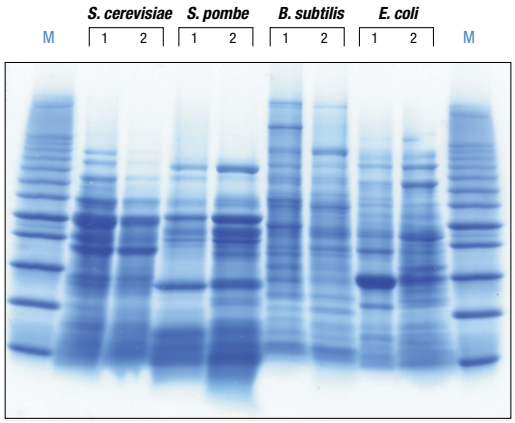

Figure 2. Thermo Scientific Y-PER Yeast Protein Extraction Reagent. Y-PER Reagent extraction of protein from two different strains each of *S. cerevisiae, S. pombe, B. subtilis and E. coli*. The samples were analyzed by 4-20% SDS-PAGE and stained with Thermo Scientific™ GelCode™ Blue Stain Reagent (Product # 24592).

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Ordering Information

Product #	Description	Pkg. Size
78990	**Y-PER-Yeast Protein Extraction Reagent** *Sufficient for 100-200g of wet cells.*	500mL
78991	**Y-PER-Yeast Protein Extraction Reagent** *Sufficient for 40-80g of wet cells.*	200mL
78999	**Y-PER-Plus Dialyzable Yeast Protein Extraction Reagent** *Sufficient for 100-200g of wet cells.*	500mL

Thermo Scientific Pierce Yeast DNA Extraction Kit

Efficient method for extracting yeast DNA in less than one hour.

The Thermo Scientific™ Pierce™ Yeast DNA Extraction Reagent Kit utilizes Y-PER Reagent to quickly and easily lyse yeast cells to release DNA for study.

This kit provides greater yields than traditional methods of DNA isolation from yeast. Typically, extraction and purification of DNA from yeast are time-consuming and labor-intensive. The yeast proteinaceous cell wall is notoriously difficult to lyse and requires harsh treatments that are time-consuming and can damage the extracted DNA. The Pierce Yeast DNA Extraction Kit protocol requires less than one hour to complete, is effective without enzymatic treatment or glass beads, and yields little to no RNA contamination regardless of RNase presence. In studies with *Saccharomyces cerevisiae*, high yields of genomic and plasmid DNA are consistently obtained. DNA purified using this kit is suitable for PCR amplification (Figure 1), bacterial transformations, restriction digestions and hybridization applications.

Highlights:

- Fast and gentle extraction – eliminates the need for glass beads or harsh enzyme treatments
- Compatible – purified DNA can be use for polymerase chain reaction (PCR) amplification, bacterial transformations, restriction digestions and hybridization applications
- Scalable – from single colonies to 500mL culture

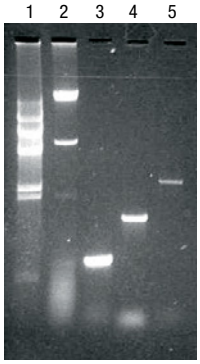


Figure 1. Extraction of yeast genomic DNA and subsequent PCR amplifications. DNA was extracted from *S. cerevisiae* strain DY150 transformed with a plasmid harboring the gene for green fluorescent protein (GFP) and purified DNA was used to amplify the chromosomal *ACT1* and *UME6* genes and the gene-encoding GFP carried on the plasmid. Lambda DNA digested with Hind III **(Lane 1)**, *S. cerevisiae* genomic DNA (smaller band approx. 5 kb corresponds to dsRNA from the yeast killer virus) **(Lane 2)**, PCR amplification of GFP from plasmid **(Lane 3)**, PCR amplification of chromosomal *ACT1* gene **(Lane 4)**, and PCR amplification of chromosomal *UME6* gene **(Lane 5)**.

Ordering Information

Product #	Description	Pkg. Size
78870	Pierce Yeast DNA Extraction Kit *Sufficient for 50 purifications from 10mL cultures.*	Kit
	Includes: Y-PER Yeast Protein Extraction Reagent	25mL
	DNA Releasing Agent A	20mL
	DNA Releasing Agent B	20mL
	Protein Removal Reagent	10mL

Thermo Scientific I-PER Insect Cell Protein Extraction Reagent

Solubilize proteins from baculovirus-infected insect cells.

The Thermo Scientific™ I-PER™ Insect Cell Protein Extraction Reagent enables gentle and effective extraction of soluble protein from *baculovirus*-infected insect cells grown in suspension or monolayer culture. The *baculovirus* insect cell expression system is an efficient and popular system for production of recombinant (eukaryotic) proteins in cell culture. Proteins expressed in baculoviral systems can be used for structural analyses, biochemical assays and a variety of other applications. I-PER Reagent maintains functionality of extracted proteins and is directly compatible with downstream applications such as protein assays, Western blotting (Figure 1) and 6xHis-tagged protein purification (Figure 2).

Highlights:

- **Gentle extraction** – optimized, mild nonionic detergent provides maximum extraction of soluble proteins from insect cells
- **Effective** – provides better protein extraction than sonication
- **Compatible** – downstream compatibility with Western blotting, 6xHis-tagged protein purification, protein assays and ion-exchange chromatography
- **Flexible** – useful for protein extraction from suspended or adherent cultured insect cells

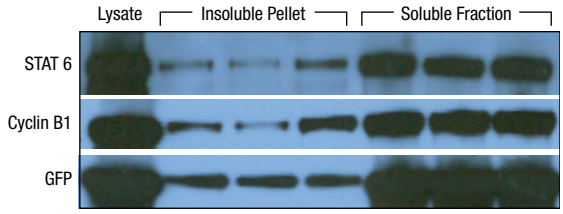


Figure 1. Thermo Scientific I-PER Reagent efficiently extracts recombinant proteins from infected Sf9 cells. I-PER Reagent extracts were prepared from infected Sf9 cells. Normalized amounts of total, insoluble and soluble protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) before Western blot analysis.

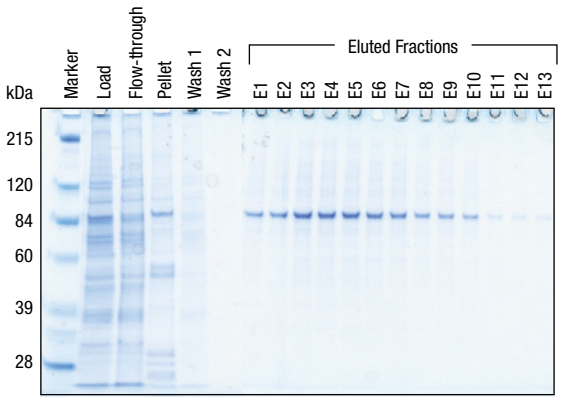


Figure 2. Affinity purification of 6xHis Cyclin B1 from Thermo Scientific I-PER Reagent extract. *Baculovirus*-infected Sf9 cells were harvested and lysed with I-PER Reagent. I-PER Reagent cell extract was directly loaded onto a nickel-chelated agarose column and purified. Protein samples were separated by SDS-PAGE and the gel was stained with GelCode Blue Stain Reagent (Product # 24590).

References

1. Katayama, T., *et. al.* (2010). *Am J Physiol Heart Circ Physiol.* **298:**H505-H514.
2. Prakash, B., *et. al.* (2008). *J. Biochem.* **144:**725-732.

Ordering Information

Product #	Description	Pkg. Size
89802	**I-PER Insect Cell Protein Extraction Reagent** *Sufficient for 5 to 20 million cultured cells per 1mL of reagent.*	250mL

Thermo Scientific P-PER Plant Protein Extraction Kit

Lyse plant leaves, stem, root, seed and flower cells without liquid nitrogen.

The Thermo Scientific™ P-PER™ Plant Protein Extraction Kit effectively extracts protein from all kinds of dry and fresh plant tissue without liquid nitrogen.

The P-PER Kit includes an organic lysing reagent and two aqueous reagents that effectively lyse plant cells and solubilize protein without harsh mechanical lysis aids, such as a mortar and pestle (Figure 1). Plant protein extracts are prepared in just 10 minutes, and protein yields exceed conventional extraction methods (e.g., freeze/grinding in liquid nitrogen) and other commercially available plant protein extraction reagents (Figure 2). The resulting protein extracts are compatible with a variety of downstream applications including the Thermo Scientific™ Pierce™ BCA Protein Assay Kit, Reducing Agent Compatible (Product # 23250), 1-D and 2-D gel electrophoresis, immunoprecipitation, Western blotting, activity assays, and protein purification.

Highlights:

- **Versatile** – works with multiple plant organs (leaf, stem, root, seed and flowers); multiple plant species (*Arabidopsis*, tobacco, maize, soybeans, peas, spinach, rice, wheat and other plant tissues); and fresh, frozen and dehydrated plant tissues
- **Convenient** – requires no liquid nitrogen/freeze-grinding, Dounce homogenization, blade-shearing or glass-bead agitation for cell disruption; however, the P-PER Kit is compatible with these alternative mechanical aids (Figure 3)
- **Compatible** – downstream applications include 1-D and 2-D gel electrophoresis (Figure 4), Western blotting, activity assays and protein affinity purifications
- **Quantifiable** – P-PER Kit extracts can be quantified using the Pierce BCA Protein Assay Kit, Reducing Agent Compatible (Product # 23250)
- **Ready to use** – protein extract does not require filtration through cheesecloth or Miracloth™ material, unlike homebrews
- **Fast** – perform plant cell lysis and protein extraction in 10 minutes
- **Recovers active protein** – assays show extracted proteins are functional

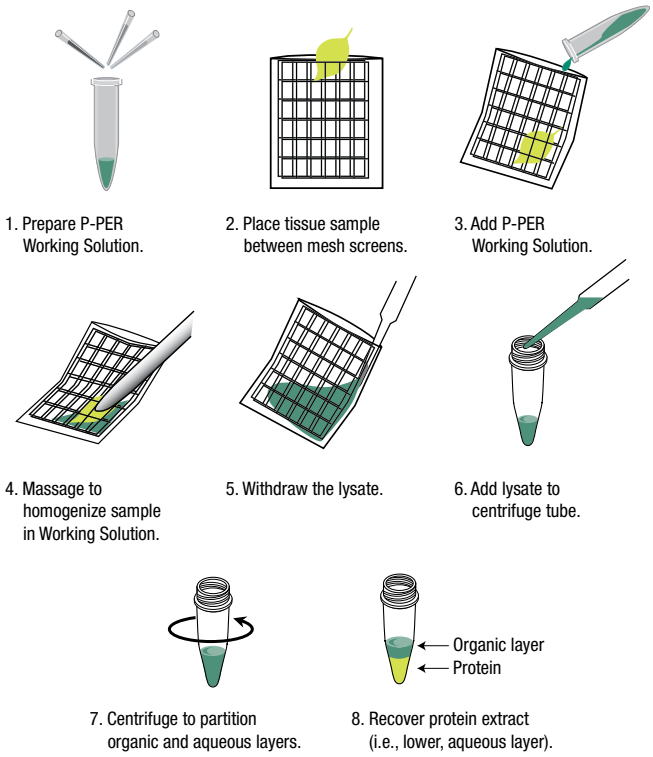


Figure 1. Thermo Scientific P-PER Plant Protein Extraction Kit protocol summary.

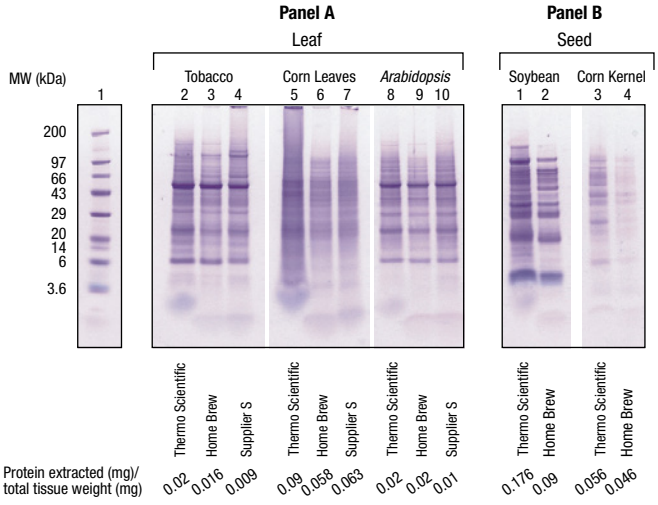


Figure 2. The Thermo Scientific P-PER Kit produces equivalent or higher levels of extracted protein than traditional and other commercial methods. Fresh leaf tissue from tobacco, maize seedlings and *Arabidopsis* were lysed and extracted according to the P-PER Kit protocol, Supplier S's protocol and a literature-based (homebrew) protocol. Samples were normalized (weight tissue/volume extract), resolved on a 10% Bis-Tris gel and stained with Imperial Protein Stain (Product # 24615). Samples were also quantified using the Pierce BCA Protein Assay Kit, Reducing Agent Compatible (Product # 23250). **Panel A. Lane 1:** molecular weight standards, **Lanes 2-4:** tobacco leaves, **Lanes 5-7:** corn leaves and **Lanes 8-10:** *Arabidopsis*. **Panel B. Lanes 1-2:** dehydrated soybean seed and **Lanes 3-4:** dehydrated corn kernel. **Note:** The Supplier S method is recommended for leaf tissue only. The extracted protein levels and the ratios of extracted protein per total plant tissue weight were determined for all samples.

Lanes:
1. Molecular Weight Marker
2. Mesh Bags
3. Glass Dounce Homogenizer
4. Liquid Nitrogen/Mortar & Pestle
5. Polytron Tissue Grinder
6. BioMasher Device (Cartagen)
7. Polypropylene Pestle (Kontes)

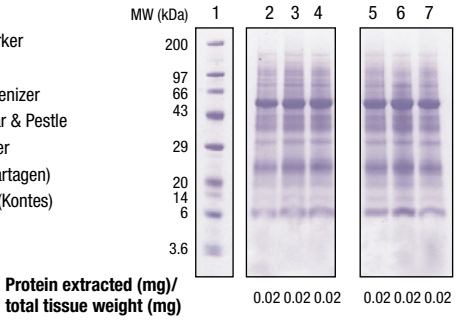


Figure 3. Thermo Scientific P-PER Reagent is compatible with common mechanical grinding aids. Fresh tobacco leaf tissue was extracted with P-PER Reagent Working Solution using common plant tissue grinding aids. Samples were normalized (weight tissue/volume extract), resolved on a 4-12% Bis-Tris gel and stained with Imperial Protein Stain (Product # 24615). Samples were also quantified using the Pierce BCA Protein Assay Kit, Reducing Agent Compatible (Product # 23250). **Lane 1:** molecular weight marker, **Lane 2:** mesh bag, **Lane 3:** Wheaton glass Dounce homogenizer, **Lane 4:** liquid nitrogen/mortar and pestle grind, **Lane 5:** Polytron tissue grinder, **Lane 6:** BioMasher™ Sample Prep Device (Cartegan) and **Lane 7:** blue polypropylene pestle (Kontes). The extracted protein levels and the ratios of extracted protein per total plant tissue weight were determined for all samples.

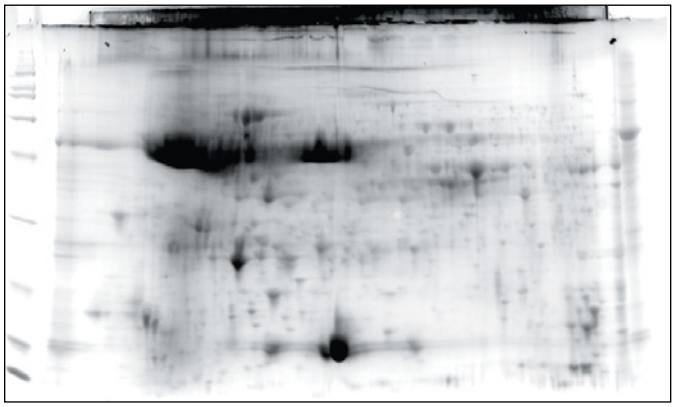


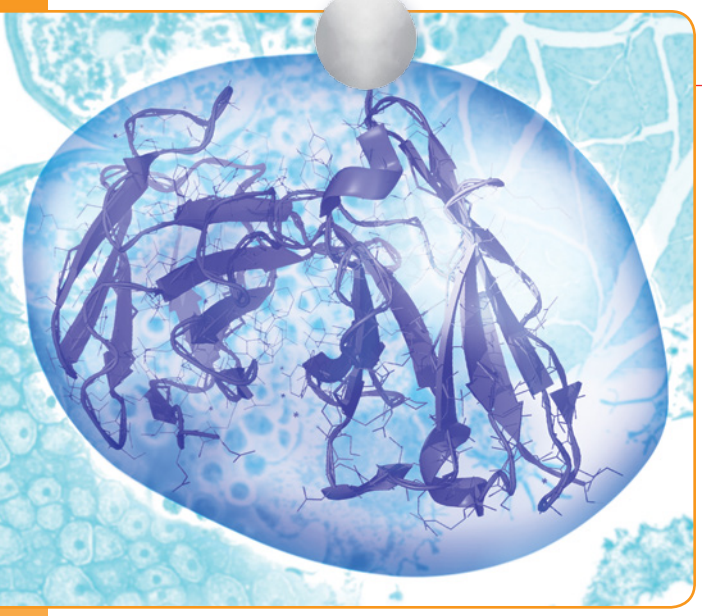
Figure 4. The Thermo Scientific P-PER Kit is compatible with 2-D gel electrophoresis. Protein was extracted from 160mg of *Arabidopsis* rosette leaves using the P-PER Kit. Samples were focused on pH 3-10 nonlinear IPG strips followed by 8-16% SDS-PAGE. (The data was provided by Dr. Sixue Chen at the Donald Danforth Plant Science Center.)

References

1. Zabka, A., *et al.* (2012). *Ann. Bot.* **110:**1581-1591.
2. Poplonska, K., *et al.* (2009). *Biol. Reprod.* **80:**572-580.
3. Shitsukawa, N., *et al.* (2007). *Plant Cell* **19:**1723-1737.

Ordering Information

Product #	Description	Pkg. Size
89803	**P-PER Plant Protein Extraction Kit**	Kit
	Sufficient for 400mg dried or 1.6g fresh plant tissue.	
	Includes: P-PER Reagent A	20mL
	P-PER Reagent B	225µL
	P-PER Reagent C	20mL
	Polypropylene Mesh Bags	20 each



introduction

Inhibition of protease and phosphatase activity

All living organisms contain proteolytic enzymes (proteases and peptidases) for protein catabolism. Protease activities are tightly regulated by compartmentalization and inhibitors to prevent indiscriminate damage to cellular proteins. Cell lysis disrupts cell membranes and organelles resulting in unregulated proteolytic activity that can reduce protein yield and function. To prevent extracted protein degradation, it is often necessary to add protease inhibitors to cell lysis reagents. Protease inhibitors function by reversibly or irreversibly binding to protease active sites. Most known proteases belong to one of four evolutionary distinct enzyme families classified by their active site functional groups. Due to the differences in the proteolytic mechanisms between these various classes of proteins (or proteases), no single compound can effectively inhibit all proteases.

In addition to proteases, phosphatases are another class of enzymes that are liberated during cell lysis. Phosphatases play a key role in regulating signal transduction pathways by removing phosphoryl groups that are transferred to proteins by kinases. Phosphorylation is one of the most common post-translational modifications on proteins with approximately 80% occurring on serine, 20% on threonine and < 1% on tyrosine residues. Identification of protein phosphorylation sites and occupancy typically requires enrichment of phosphoproteins or phosphopeptides before MS analysis. These techniques require intact phospho group modifications for enrichment, which requires the use of phosphatase inhibitors to prevent dephosphorylation.

Most researchers use a mixture or "cocktail" of several different inhibitor compounds to ensure that protein extracts do not degrade before analysis of targets of interest. Protease inhibitors are nearly always needed, while phosphatase inhibitors are required only when investigating phosphorylation states (activation states). Particular research experiments may require the use of single inhibitors or customized mixtures, but most protein work is best served by using a broad-spectrum protease inhibitor cocktail.

Other considerations for protein function and stability during cell lysis

When the goal of cell lysis is to purify or test the function of a particular protein(s), special attention must be given to the effects that the lysis reagents have on the stability and function of the target proteins. Certain detergents will inactivate the function of particular enzymes or disrupt protein complexes. Downstream analysis of extracted/purified proteins may also require salt and/or detergent removal in order to study proteins of interest or maintain long-term stability of the extracted protein.

Thermo Scientific Protease and Phosphatase Inhibitor Cocktails

Inhibit protease activity and/or protect against specific phosphatase activities during cell lysis and protein extraction with these ready-to-use inhibitor solutions or tablets.

Thermo Scientific™ Halt™ Inhibitor Cocktails are ready-to-use, 100X stock solutions of broad-spectrum protease and phosphatase inhibitors. Simply pipette the volume of concentrated cocktail your sample requires to ensure complete protection of the resulting protein extract. Halt Protease Inhibitor Cocktails and Combined Protease and Phosphatase Inhibitor Cocktails are available in both EDTA and EDTA-free formulations. Halt Inhibitor Cocktails are available in single-use format (24 x 100µL microtubes) and 1mL, 5mL and 10mL package sizes.

Thermo Scientific™ Pierce™ Protease, Phosphatase, and Combined Protease and Phosphatase Inhibitor Tablets are quick-dissolving tablets conveniently provided in vials and may be reconstituted before extract preparation for maximum protection. The formulations are available with or without EDTA. Each Pierce Inhibitor Tablet is sufficient for either 10mL or 50mL of solution.

Highlights:

- **Multiple package sizes** – liquid cocktails are available in 100µL single-use format or 1, 5 and 10mL pack sizes; tablets come in two sizes – for 10 or 50mL volumes to accommodate different volume/pricing needs
- **Convenient** – the refrigerator-stable, 100X liquid or tablet format is more effective and easier to use than individual inhibitors; just pipette the amount you need, or add a tablet to a 10 or 50mL solution
- **No proprietary ingredients** – components are fully disclosed
- **Two popular formulations** – available with or without EDTA; EDTA-free formulation ensures compatibility with isoelectric focusing or His-tag purification
- **Complete protection** – all-in-one formulations contain both protease and phosphatase inhibitors (combined cocktail only)
- **Compatible** – use with Thermo Scientific™ Pierce™ Cell Lysis Buffers or nearly any other commercial or homemade detergent-based lysis reagent; also works neat or diluted with standard protein assays, including BCA and coomassie (Bradford)

Protease and phosphatase inhibitors are essential components of most cell lysis and protein extraction procedures. These inhibitors block or inactivate endogenous proteolytic and phospholytic enzymes that are released from subcellular compartments during cells lysis.

Our protease inhibitor cocktails and tablets target serine, cysteine and aspartic acid proteases, and aminopeptidases. Metalloproteases are inhibited by the optional addition of EDTA (available in a separate vial in the liquid format and included in the tablet format). The phosphatase inhibitor cocktails and tablets target serine/threonine and tyrosine phosphatases. These inhibitors are ideal for the protection of proteins during extraction or lysate preparation from cultured cells, animal tissues, plant tissues, yeast or bacteria.

For further savings and convenience, combined Thermo Scientific Protease and Phosphatase Inhibitor Cocktails and Tablets are offered. These prevent protein degradation and preserve phosphorylation simultaneously, providing complete protection in a single solution or tablet.

All Halt Inhibitor Cocktails and Pierce Inhibitor Tablets are compatible with Thermo Scientific™ Pierce™ Protein Extraction Reagents and most homemade and commercial cell lysis solutions.

▸ Learn more at thermofisher.com/proteinextraction

Table 1. Components present in the Thermo Scientific Halt Inhibitor Cocktails and Thermo Scientific Pierce Protease and Phosphatase Inhibitor Tablets.

Inhibitor Component	Target (mechanism)	Protease Liquid Cocktails and Tablets	Phosphatase Liquid Cocktails and Tablets	Combined Protease and Phosphatase Liquid Cocktails and Tablets
AEBSF•HCl	Serine Proteases (irreversible)	✖		
Aprotinin	Serine Protease (reversible)	✖		✖
Bestatin	Aminopeptidase (reversible)	✖		✖
E-64	Cysteine (irreversible)	✖		✖
Leupeptin	Serine and Cysteine Proteases (reversible)	✖		✖
Pepstatin	Aspartic Acid Proteases (reversible)	✖		
EDTA†	Metalloproteases (reversible)	✖		✖
Sodium Fluoride	Serine/Threonine and Acidic Phosphatases		✖	✖
Sodium Orthovanadate	Tyrosine and Alkaline Phosphatases		✖	✖
β-glycero-phosphate	Serine/Threonine Phosphatase		✖	✖
Sodium Pyrophosphate	Serine/Threonine Phosphatase		✖	✖

† *EDTA not in EDTA-free formulations.*

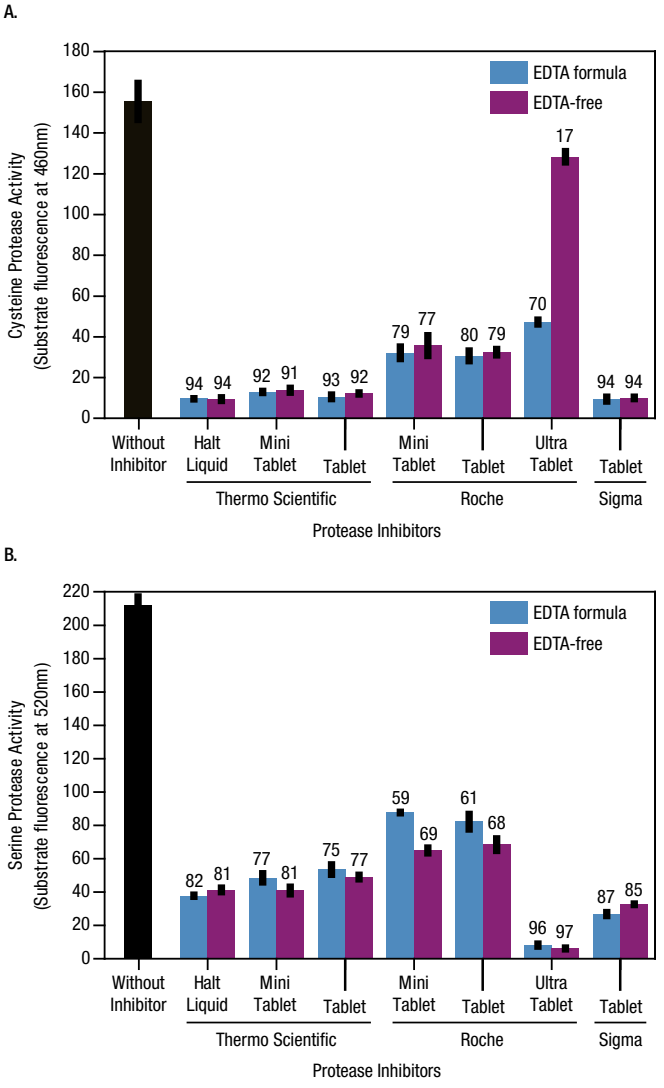


Figure 1. Comparison of commercially available protease inhibitor cocktails and tablets. Pancreatic extract (50µL; 1µg/µL protein) or trypsin (25µL, 0.1 units/µL) was incubated with a quenched-fluorescent, protease-cleavable substrate for cysteine (**A**) or serine proteases (**B**) in the presence or absence of commercially available protease inhibitors with EDTA-containing (blue) or EDTA-free (purple) formulations. Reactions were incubated for two hours at 37°C and the fluorescence determined at indicated detecting emissions. The percent protease inhibition is shown for each protease inhibitor formulation.

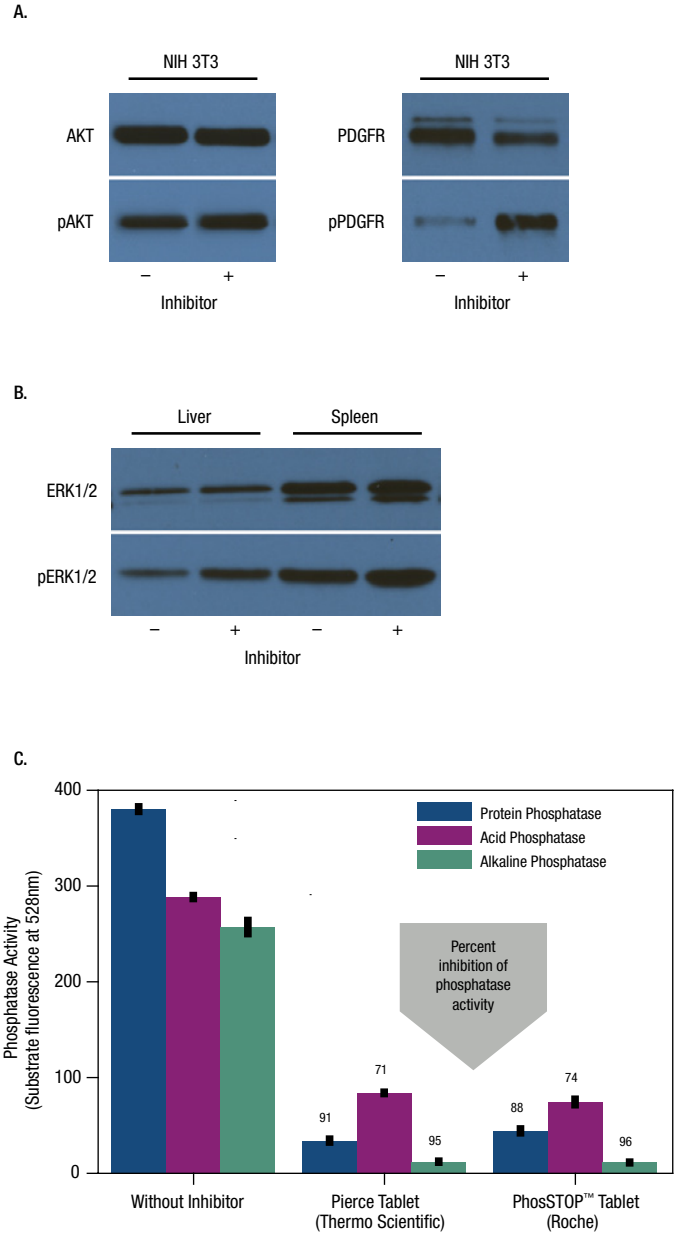


Figure 2. Protein phosphorylation is preserved in cell and tissue extracts. Relative levels of total and phosphorylated protein from extracts prepared in the absence or presence of phosphatase inhibitors were determined by Western blot analysis. (**A**): AKT and PDGFR in serum-starved, PDGF-stimulated (100ng/mL) NIH 3T3 cell extracts. (**B**): ERK1/2 in liver and spleen tissue extracts. (**C**): the degree of inhibition for protein, acid and alkaline phosphatase activity was determined in mouse brain extract after treatment with Pierce Phosphatase Inhibitor Tablets or another commercially available phosphatase inhibitor tablet. Percent inhibition is indicated.

Ordering Information

Product #	Description	Pkg. Size
Protease Inhibitor Cocktails		
78430	Halt Protease Inhibitor Single-Use Cocktail (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
87786	Halt Protease Inhibitor Cocktail (100X) *Sufficient for 100mL of sample.*	1mL
78429	Halt Protease Inhibitor Cocktail (100X) *Sufficient for 500mL of sample.*	5mL
78438	Halt Protease Inhibitor Cocktail (100X) *Sufficient for 1L of sample.*	10mL
78425	Halt Protease Inhibitor Single-Use Cocktail, EDTA-free (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
87785	Halt Protease Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 100mL of sample.*	1mL
78437	Halt Protease Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 500mL of sample.*	5mL
78439	Halt Protease Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 1L of sample.*	10mL
Phosphatase Inhibitor Cocktails		
78428	Halt Phosphatase Inhibitor Single-Use Cocktail *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
78420	Halt Phosphatase Inhibitor Cocktail *Sufficient for 100mL of sample.*	1mL
78426	Halt Phosphatase Inhibitor Cocktail *Sufficient for 500mL of sample.*	5 x 1mL
78427	Halt Phosphatase Inhibitor Cocktail *Sufficient for 1L of sample.*	10mL

Product #	Description	Pkg. Size
Combined Protease and Phosphatase Inhibitors Cocktails		
78442	Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
78440	Halt Protease and Phosphatase Inhibitor Cocktail (100X) *Sufficient for 100mL of sample.*	1mL
78444	Halt Protease and Phosphatase Inhibitor Cocktail (100X) *Sufficient for 500mL of sample.*	5 x 1mL
78446	Halt Protease and Phosphatase Inhibitor Cocktail (100X) *Sufficient for 1L of sample.*	10mL
78443	Halt Protease and Phosphatase Inhibitor Single-Use Cocktail, EDTA-free (100X) *Sufficient for 240mL of sample in 10mL increments.*	24 x 100µL
78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 100mL of sample.*	1mL
78445	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) *Sufficient for 500mL of sample.*	5 x 1mL
78447	Halt Protease and Phosphatase Inhibitor Cocktail, DTA-free (100X) *Sufficient for 1L of sample.*	10mL

Ordering Information

Product #	Description	Pkg. Size
Protease Inhibitor Tablets		
88665	Pierce Protease Inhibitor Mini Tablets *Sufficient for 300mL of sample.*	30 tablets
88265	Pierce Protease Inhibitor Tablets *Sufficient for 1000mL of sample.*	20 tablets
88666	Pierce Protease Inhibitor Mini Tablets, EDTA-free *Sufficient for 300mL of sample.*	30 tablets
88266	Pierce Protease Inhibitor Tablets, EDTA-free *Sufficient for 1000mL of sample.*	20 tablets

Product #	Description	Pkg. Size
Phosphatase Inhibitor Tablets		
88667	Pierce Phosphatase Inhibitor Mini Tablets *Sufficient for 200mL of sample.*	20 tablets
Combined Protease and Phosphatase Inhibitors Tablets		
88668	Pierce Protease and Phosphatase Inhibitor Mini Tablets *Sufficient for 200mL of sample.*	20 tablets
88669	Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free *Sufficient for 200mL of sample.*	20 tablets

Thermo Scientific Protease Inhibitors (Individual)

Individual protease inhibitor components for experiment customization.

Thermo Scientific™ Protease Inhibitors are small packages of individual protease inhibitor peptides and compounds for customized formulation or modification of protease inhibitor cocktails.

The individual reagents include AEBSF, aprotinin, bestatin, E64, leupeptin, pepstatin A and PMSF. Although Halt Protease Inhibitor Cocktails provide convenient and optimized broad-spectrum protease inhibition for routine cell lysis and assay needs, certain applications require a more customized approach. By offering the individual, high-quality reagents used to make our cocktail products, we provide you with the tools needed to apply a protease inhibitor individually, supplement a cocktail or combine several components to make customized protease inhibitor cocktails for specialized applications.

Highlights:

- **Convenient** – high-quality reagents provided in convenient, affordable package sizes
- **Customizable** – purchase individually to prepare customized protease inhibitor cocktails or supplement Halt Protease Inhibitor Cocktails, whose formulations are fully disclosed, to achieve desired concentrations of specific component reagents

Table 1. General properties of protease inhibitors.

Protease Inhibitor	MW	Protease Family Targeted	Inhibitor Type	Solubility (Solvent)
AEBSF•HCl	239.5	Serine proteases	Irreversible	200mg/mL (H_2O)
Aprotinin	6511.5	Serine proteases	Reversible	10mg/mL (H_2O)
Bestatin	308.38	Amino-peptidases	Reversible	5mg/mL (Methanol)
E-64	357.4	Cysteine proteases	Irreversible	20mg/mL (50% Ethanol)
Leupeptin	475.6	Serine and cysteine proteases	Reversible	1mg/mL (H_2O)
Pepstatin A	685.9	Aspartic acid proteases	Reversible	1mg/mL (Methanol)

Ordering Information

Product #	Description	Pkg. Size
78431	AEBSF Protease Inhibitor	100mg
78432	Aprotinin Protease Inhibitor	25mg
78433	Bestatin Protease Inhibitor	10mg
78434	E-64 Protease Inhibitor	10mg
78435	Leupeptin Protease Inhibitor	50mg
78436	Pepstatin A Protease Inhibitor	25mg

Thermo Scientific PMSF (phenylmethylsulfonyl fluoride)

Binds to active serine residues in trypsin, chymotrypsin, thrombin and papain.

Thermo Scientific™ PMSF is a protease inhibitor that reacts with serine residues to inhibit trypsin, chymotrypsin, thrombin and papain. PMSF is an abbreviation for phenylmethylsulfonyl fluoride, the most common chemical name for this small compound. In addition to inhibiting serine proteases, PMSF will also inhibit cysteine proteases, like papain (reversible by DTT treatment), and mammalian acetylcholinesterase. Because PMSF has limited water solubility, it must be dissolved in a small amount of solvent, such as ethanol, methanol or isopropyl alcohol before addition to a buffer.

Highlights:

- **Specific** – targets serine proteases, including trypsin, chymotrypsin, thrombin and papain
- **Effective** – achieves inhibition at concentrations as low as 0.1 to 1mM final concentration

Properties of PMSF:

- Synonyms: alpha-Toluenesulfonyl fluoride; Benzylsulfonyl fluoride; Phenylmethylsulfonyl fluoride
- Formula: $C_6H_5CH_2SO_2F$
- Molecular weight: 174.19
- CAS Number: 329-98-6; EC Number: 206-350-2
- R/S Codes: R 25-34; S 26-36/37/39-45
- Storage conditions: room temperature in a dry place protected from light
- Working solution: dissolve PMSF in isopropanol at 1.74mg/mL (10mM); store in aliquots at -20°C

Ordering Information

Product #	Description	Pkg. Size
36978	PMSF Protease Inhibitor	5g

Thermo Scientific Protein Stabilizing Cocktail

Preserve the function and activity of enzymes for long-term storage.

The Thermo Scientific™ Protein Stabilizing Cocktail is a versatile stabilizing solution that increases the shelf-life of purified or partially purified proteins during routine storage. This proprietary formulation of low-molecular weight, naturally occurring molecules helps protect proteins from environmental stresses that can otherwise lead to enzyme inactivation, aggregation and freeze-thaw damage.

Protein Stabilizing Cocktail is provided as an easy-to-pipette, buffered 4X concentrate. Solutions of enzymes and other proteins to which the cocktail has been added may be refrigerated or frozen for storage without losing activity or function. Although the degree of stabilization is protein-specific, the cocktail significantly stabilizes most proteins compared with conventional buffer alone. Protein Stabilizing Cocktail is nontoxic and does not destabilize biomolecules; however, all cocktail components can be removed by dialysis or desalting before use in downstream assays, if desired.

Highlights:

- **Better stabilization** – significantly better stabilization of enzymes than ordinary buffers and does not destabilize biomolecules in downstream assays
- **Protects proteins from environmental stresses** – reducing enzyme inactivation, aggregation and freeze-thaw damage
- **Convenient** – components are low-molecular weight and fully dialyzable
- **Easy to use** – low-viscosity reagent is easier to pipette than 50% glycerol
- **Versatile** – protein classes tested include kinases, phosphatases, peroxidases, restriction enzymes, luciferases, cytokines and antibodies

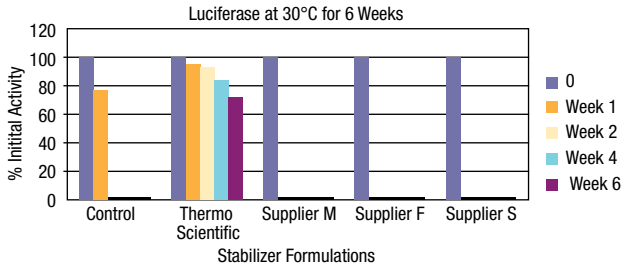
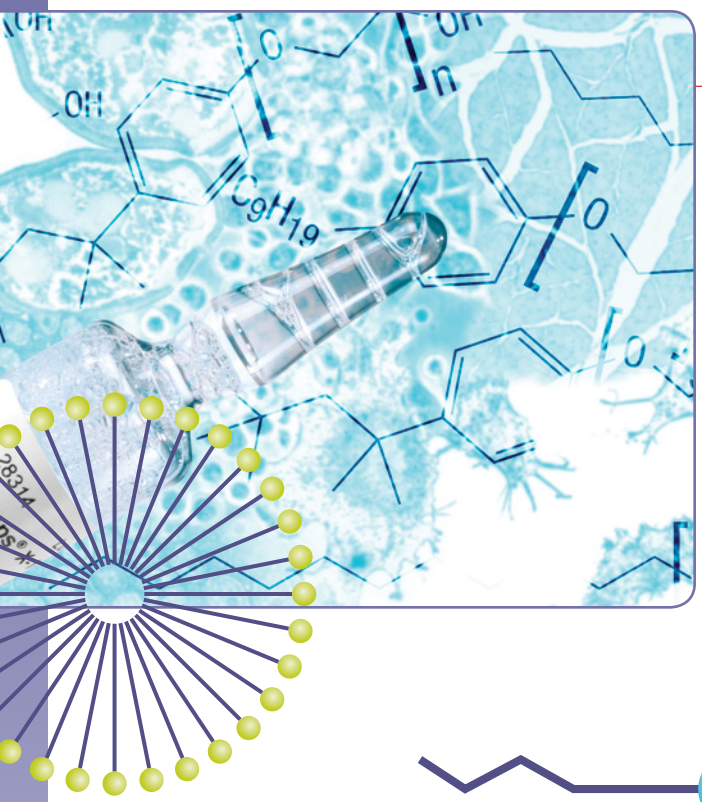


Figure 1. Performance comparison of protein activity stabilizers. Luciferase activity was assessed upon storage at 50µg/mL at 30°C in Protein Stabilizing Cocktail (Thermo Scientific) and three other commercially available stabilizer products. Fluorescence was measured at time 0 (dark bars) and after 1, 2, 4 and 6 weeks of storage. Luciferase stored in Protein Stabilizing Cocktail maintained 85% of its original activity after four weeks compared to a Tris-buffered saline formulation (control) and other suppliers' stabilizing agents, which were completely inactive after two weeks.

Ordering Information

Product #	Description	Pkg. Size
89806	Protein Stabilizing Cocktail, 4X Concentrated Solution *Sufficient reagent to make 40mL of storage solution.*	10mL



overview

Detergents are a class of molecules whose unique properties enable manipulation (disruption or formation) of hydrophobic-hydrophilic interactions among molecules in biological samples. In life science applications, detergents are used for cell lysis, protein solubilization and denaturation, or to reduce background in certain applications.

Detergents are amphipathic molecules, meaning they contain both a nonpolar "tail" having aliphatic or aromatic character and a polar "head." The ionic character of the polar head group forms the basis for broad classification of detergents; they may be ionic (charged, either anionic or cationic), nonionic (uncharged) or zwitterionic (having both positively and negatively charged groups but with a net charge of zero). These different properties can be exploited for various protein methods.

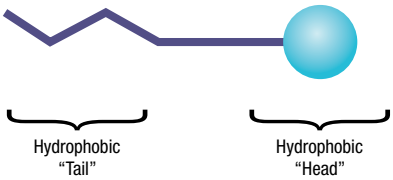


Figure 1. Generic structure of a detergent molecule.

Types of detergents

In protein biology, detergents can be described as denaturing or non-denaturing. Denaturing detergents are generally ionic, such as sodium dodecyl sulfate (SDS). Non-denaturing detergents can be divided into nonionic detergents and zwitterionic detergents.

Ionic detergents have a positive (cationic) or negative (anionic) hydrophilic head that can be utilized for the complete disruption of cellular membranes and the denaturation of proteins. Ionic detergents bind to and mask the native charge of proteins, conferring the same overall charge as the detergent itself. SDS, a common anionic detergent, is used in gel electrophoresis and Western blotting.

Nonionic detergents have an uncharged hydrophilic head that can disrupt lipid-protein or lipid-lipid interactions, but have limited effects on protein-protein interactions. They are classified as non-denaturing detergents and can be used for isolating biologically active membrane proteins. Triton X-100 and Tween™-20 detergents are popular nonionic detergents that are used for protein extraction or to reduce nonspecific binding in immunoassays, respectively.

Zwitterionic detergents possess a net zero charge arising from the presence of equal numbers of positively and negatively charged chemical groups in the hydrophilic head. These detergents protect the native state of proteins without altering the native charge of the protein molecules and can disrupt protein-protein interactions. They can be used for applications such as gel electrophoresis or Western blotting. CHAPS detergent is an example of a zwitterionic detergent.

Detergent properties

Detergents interact with proteins in their micelle state. Like the components of biological membranes, detergents have hydrophobic-associating properties as a result of their nonpolar tail groups. Nevertheless, detergents themselves are water-soluble. Consequently, detergent molecules allow the dispersion (miscibility) of water-insoluble, hydrophobic compounds into aqueous media, including the extraction and solubilization of membrane proteins.

Detergents at low concentration in aqueous solution form a monolayer at the air-liquid interface. At higher concentrations, detergent monomers aggregate into structures called micelles. A micelle is a thermodynamically stable colloidal aggregate of detergent monomers wherein the nonpolar ends are sequestered inward, avoiding exposure to water, and the polar ends are oriented outward in contact with the water.

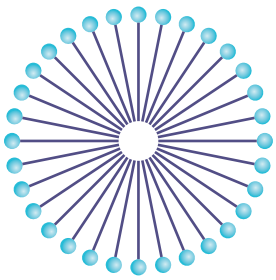


Figure 2. Idealized structure of a detergent micelle.

Both the number of detergent monomers per micelle (aggregation number) and the range of detergent concentration above which micelles form (called the critical micelle concentration, CMC) are properties specific to each particular detergent (see Table 1). The critical micelle temperature (CMT) is the lowest temperature at which micelles can form. The CMT corresponds to what is known as the cloud point because detergent micelles form crystalline suspensions at temperatures below the CMT and are clear again at temperatures above the CMT.

Detergent properties are affected by experimental conditions such as concentration, temperature, buffer pH and ionic strength, and the presence of various additives. For example, the CMC of certain nonionic detergents decreases with increasing temperature, while the CMC of ionic detergents decreases with addition of counter ion as a result of reduced electrostatic repulsion among the charged head groups. In other cases, additives such as urea effectively disrupt water structure and cause a decrease in detergent CMC. Generally, dramatic increases in aggregation number occur with increasing ionic strength.

Table 1. Properties of common detergents.

Detergent	Description	Aggregation Number	Micelle MW	MW	CMC (mM)	CMC % w/v	Cloud Point (°C)	Dialyzable
Triton X-100	Nonionic	140	90,000	647	0.24	0.0155	64	No
Triton X-114	Nonionic	—	—	537	0.21	0.0113	23	No
NP-40	Nonionic	149	90,000	617	0.29	0.0179	80	No
Brij-35	Nonionic	40	49,000	1225	0.09	0.1103	> 100	No
Brij-58	Nonionic	70	82,000	1120	0.077	0.0086	> 100	No
Tween-20	Nonionic	—	—	1228	0.06	0.0074	95	No
Tween-80	Nonionic	60	76,000	1310	0.012	0.0016	—	No
Octyl Glucoside	Nonionic	27	8,000	292	23-25	0.6716-0.7300	> 100	Yes
Octylthio Glucoside	Nonionic	—	—	308	9	0.2772	> 100	Yes
SDS	Anionic	62	18,000	288	6-8	0.1728-2304	> 100	Yes
CHAPS	Zwitterionic	10	6,149	615	8-10	0.4920-0.6150	> 100	Yes

Learn more at thermofisher.com/detergents

Importance of detergent purity

There are several key factors to consider when preparing formulations using detergents: viscosity and purity of the detergent, and stability of the protein of interest. Detergents in their neat state are highly viscous and difficult to accurately measure and dispense. Therefore, it is advantageous to purchase or prepare precisely diluted detergent stock solutions of 10-30%, depending on the concentration needed for the final formulation.

Although detergents are available from many commercial sources and used routinely in research laboratories, the importance of detergent purity and stability is not widely appreciated. Detergents often contain trace impurities carried over from their production. Some of these impurities, especially peroxides, will reduce protein activity. In addition, several types of detergents oxidize readily when exposed to the air or UV-light, causing them to lose their properties and potency as solubilizing agents. For life science and diagnostic applications, we highly recommend using detergents that have been purified to remove any contaminating peroxides and carbonyls. Most suppliers of purified detergents will report the level of these two contaminants in their documentation.

Another important consideration when using detergents is the downstream application. However necessary and beneficial the use of detergent may have been for initial cell lysis or membrane protein extractions, subsequent analysis or experiments with the extracted proteins may require removal of some or all of the detergent(s). For example, although many water-soluble proteins are functional in detergent-solubilized form, membrane proteins are often modified and inactivated by detergent solubilization as a result of the disruption of native lipid interactions. In some such cases, membrane protein function is restored when they are reconstituted into bilayer membranes by replacement of detergent with phospholipids or other membrane-like lipid mixtures.

The function of an individual protein can be studied in isolation if it is first purified and then reconstituted into an artificial membrane (although recovery of native orientation in the membrane is a major challenge). Even where restoration of protein function is not critical, detergent concentration may have to be decreased in a sample to make it compatible with protein assays or gel electrophoresis. In addition, even low detergent concentrations contaminate instruments and interfere with column binding, elution and ionization when analyzing protein or peptide samples using MS.

We provide a range of high-purity, low-peroxide, surfactant solutions and detergent solids for use in cell lysis reagent formulation, protein solubilization procedures, wash buffers for ELISA, and other protein research methods.

Our Thermo Scientific™ Pierce™ Surfact-Amps™ Detergents are highly purified, precisely diluted (10%) formulations that are ideal for applications or assays that are sensitive to contaminants that are present in unpurified detergents. We test every batch to ensure that our detergents contain <1.0 µeq/mL peroxides and carbonyls and we package them under nitrogen, to prevent oxidization during storage.

Detergent removal

The detergents and surfactants used to prepare protein and peptide samples can interfere with analysis by ELISA, isoelectric focusing and MS. Removing detergents from peptide samples is especially challenging and critical for MS analysis because even low concentrations of detergents will contaminate instruments and interfere with column binding, elution and peptide ionization.

Detergent removal can be attempted in a number ways. Dialysis is effective for removal of detergents that have very high CMCs and/or small aggregation numbers, such the N-octyl glucoside formulations. Detergents with low CMCs and large aggregation numbers cannot be dialyzed because most of the detergent molecules will be in micelles that are too large to diffuse through the pores of the dialysis membrane; only excess monomer can be dialyzed. Ion exchange chromatography using appropriate conditions to selectively bind and elute the proteins of interest is another effective way to remove detergent. Sucrose density gradient separation also can be used. However, all these methods can be somewhat labor- and/or time-intensive or detergent-specific.

Thermo Fisher Scientific has developed a proprietary resin for efficient and effective detergent extraction. The Thermo Scientific™ Pierce™ Detergent Removal Products specifically bind a wide variety of detergents and surfactants that are commonly used in protein extraction and biological sample preparation and provide an especially convenient and rapid format for treating protein and peptide solutions to remove interfering detergents before downstream analysis by MS and other techniques.

Our detergent removal resins are provided in convenient spin column or plate formats that quickly and efficiently remove ionic, nonionic and/or zwitterionic detergents from protein or peptide samples to improve compatibility with downstream applications.

Thermo Scientific Surfact-Amps Tween-20 Detergent

Provides purity, quality and stability in a convenient 10% solution.

Thermo Scientific™ Surfact-Amps™ Tween™-20 Detergent Solution is highly-purified Tween-20 detergent stabilized as a 10% solution in 10mL glass ampules or three sizes of HDPE-plastic bottles.

This easy-to-use 10% (w/v) solution of purified Tween-20 detergent can be used in routine and high-demand protein research methods and molecular biology techniques. Tween-20 detergent is a nonionic polyoxyethylene surfactant that is most frequently added to PBS or TBS wash buffers for ELISA, Western blotting and other immunoassay methods. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or non-leaching HDPE bottles, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

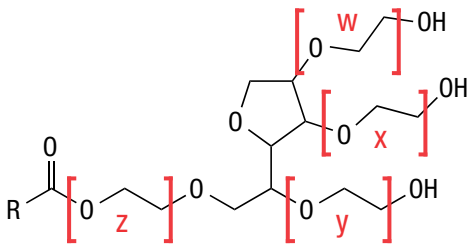
- **Accurate** – precise 10% detergent solution in ultrapure water
- **Easy to use** – solution is simple to dispense and dilute
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged under inert nitrogen gas in glass ampules or HDPE bottles

Properties of Tween-20 Detergent:

- Molecular Weight: 1228g
- Detergent Class: Nonionic
- Aggregation Number: Unknown
- Micelle Molecular Weight: Unknown
- Critical Micelle Concentration (CMC): 0.06mM (0.0074%, w/v)
- Cloud Point: 95°C
- Dialyzable: No

Specifications for Surfact-Amps Tween-20 Detergent Solution:

- Visual: Clear, light yellow liquid, free of particulates
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference



Tween-20 Detergent
w + x + y + z = 20
R = $CH_2(CH_2)_9CH_3$
MW 1228

Table 1. Purity comparison of Tween-20 detergents.

Manufacturer/Brand	Peroxide Concentration (ueq/mL)	Carbonyl Concentration (ueq/mL)
Thermo Scientific	≤ 0.01	≤ 0.32
Amresco	0.598	0.399
Anatrace	≤ 0.01	≤ 0.32
G-Bioscience	0.718	≤ 0.32
Millipore EMD	0.037	≤ 0.32
Roche	0.279	0.445

Ordering Information

Product #	Description	Pkg. Size
28320	Surfact-Amps Tween-20 Detergent Solution	6 x 10mL
85113	Surfact-Amps Tween-20 Detergent Solution	50mL
85114	Surfact-Amps Tween-20 Detergent Solution	250mL
85115	Surfact-Amps Tween-20 Detergent Solution	500mL
28321	Surfact-Amps Tween-20 Detergent Solution	1L

Thermo Scientific Surfact-Amps Tween-80 Detergent Solution

Provides purity, quality and stability in a convenient 10% solution.

Thermo Scientific™ Surfact-Amps™ Tween™-80 Detergent Solution is highly purified Tween-80 detergent stabilized as a 10% solution in 10mL glass ampules or two sizes of HDPE-plastic bottles.

This easy-to-use 10% (w/v) solution of purified Tween-80 detergent can be used in routine and high-demand protein research methods and molecular biology techniques. Tween-80 detergent is a nonionic polyoxyethylene surfactant that is most frequently added to PBS or TBS wash buffers for ELISA, Western blotting and other immunoassay methods. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or non-leaching HDPE bottles, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

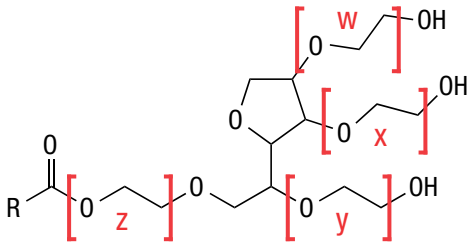
- **Accurate** – precise 10% detergent solution in ultrapure water
- **Easy to use** – solution is simple to dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged under inert nitrogen gas in glass ampules or HDPE bottles

Properties of Tween-80 Detergent:

- Molecular Weight: 1310g
- Detergent Class: Nonionic
- Aggregation Number: 60
- Micelle Molecular Weight: 76,000g
- Critical Micelle Concentration (CMC): 0.012mM (0.0016%, w/v)
- Cloud Point: Unknown
- Dialyzable: No

Specifications for Surfact-Amps Tween-80 Detergent Solution:

- Visual: Slightly hazy to cloudy, colorless solution, free of particulates; may form layers
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference



Tween-80 Detergent
w + x + y + z = 20
R = $CH_2(CH_2)_5CH_2CH{=}CHCH_2(CH_2)_6CH_3$
MW 1310

Table 1. Purity comparison of Tween-80 detergents.

Manufacturer/Brand	Peroxide Concentration (ueq/mL)	Carbonyl Concentration (ueq/mL)
Thermo Scientific	≤ 0.005	≤ 0.6
Anatrace	0.02385	≤ 0.6
G-Bioscience	0.00771	≤ 0.6

Ordering Information

Product #	Description	Pkg. Size
28328	**Surfact-Amps Tween-80 Detergent Solution**	6 x 10mL
28329	**Surfact-Amps Tween-80 Detergent Solution**	50mL
28230	**Surfact-Amps Tween-80 Detergent Solution**	500mL

Thermo Scientific Surfact-Amps Triton X-100 Detergent Solution

Provides purity, quality and stability in a convenient 10% solution.

Thermo Scientific™ Surfact-Amps™ Triton™ X-100 Detergent Solution is highly purified Triton X-100 detergent stabilized as a 10% solution in 10mL glass ampules or three sizes of HDPE-plastic bottles.

This easy-to-use 10% (w/v) solution of purified Triton X-100 detergent is ideal for use in both routine and high-demand protein research methods and molecular biology techniques. Triton X-100 detergent is a nonionic polyoxyethylene surfactant that is most frequently used as a component of cell lysis buffers or other solutions intended to extract and solubilize proteins. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Detergent Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or non-leaching HDPE bottles, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

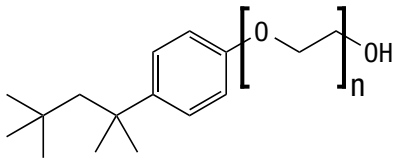
- **Accurate** – precise 10% detergent solution in ultrapure water
- **Easy to use** – solution is simple to dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged under inert nitrogen gas in glass ampules or HDPE bottles

Properties of Triton X-100 Detergent:

- Molecular Weight: 647g
- Detergent Class: Nonionic
- Aggregation Number: 140
- Micelle Molecular Weight: 90,000g
- Critical Micelle Concentration (CMC): 0.24mM (0.0155%, w/v)
- Cloud Point: 64°C
- Dialyzable: No

Specifications for Surfact-Amps Triton X-100 Detergent Solution:

- Visual: Clear to slightly hazy, colorless solution, free of particulate matter
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference



Triton X-100 Detergent
n = 9-10
MW 647

Table 1. Purity comparison of Triton X-100 detergents.

Manufacturer/Brand	Peroxide Concentration (ueq/mL)	Carbonyl Concentration (ueq/mL)
Thermo Scientific	≤ 0.20	≤ 0.20
Amresco	≤ 0.20	≤ 0.20
Anatrace	≤ 0.20	0.333
G-Bioscience	≤ 0.20	≤ 0.20
Millipore EMD	≤ 0.20	≤ 0.20
Roche	≤ 0.20	0.253
Sigma	≤ 0.20	0.355

Ordering Information

Product #	Description	Pkg. Size
28314	**Surfact-Amps Triton X-100 Detergent Solution**	6 x 10mL
85111	**Surfact-Amps Triton X-100 Detergent Solution**	50mL
85112	**Surfact-Amps Triton X-100 Detergent Solution**	250mL
28313	**Surfact-Amps Triton X-100 Detergent Solution**	1L

Thermo Scientific Surfact-Amps Triton X-114 Detergent Solution

Purity, quality and stability in a convenient 10% solution.

Thermo Scientific™ Surfact-Amps™ Triton™ X-114 Detergent Solution is highly purified Triton X-114 detergent stabilized and supplied as a 10% solution in 10mL glass ampules.

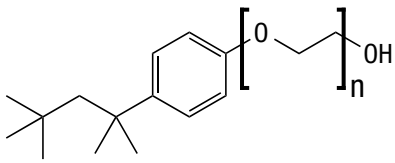
This easy-to-use 10% (w/v) solution of purified Triton X-114 detergent is ideal for use in both routine and high-demand protein research methods and molecular biology techniques. Triton X-114 detergent is a nonionic polyoxyethylene surfactant that is most frequently used as a component of cell lysis buffers. In certain conditions, this detergent phase-separates in aqueous solutions, a property that has been used to separate hydrophilic and hydrophobic (membrane) proteins. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

- **Accurate** – precise 10% detergent solution in ultrapure water
- **Easy to use** – solution is simple to dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged in glass ampules under inert nitrogen gas

Properties of Triton X-114 Detergent:

- Molecular Weight: 537g
- Detergent Class: Nonionic
- Aggregation Number: Unknown
- Micelle Molecular Weight: Unknown
- Critical Micelle Concentration (CMC): 0.21mM (0.0113%, w/v)
- Cloud Point: 23°C
- Dialyzable: No



Triton X-114 Detergent
n = 7-8
MW 537

Specifications for Surfact-Amps Triton X-114 Detergent Solution:

- Visual: Slightly hazy to cloudy, colorless solution, free of particulates; may form layers
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference

Ordering Information

Product #	Description	Pkg. Size
28332	Surfact-Amps Triton X-114 Detergent Solution	6 x 10mL

Thermo Scientific Surfact-Amps NP-40 Detergent Solution

Provides purity, quality and stability in a convenient 10% solution.

Thermo Scientific™ Surfact-Amps™ NP-40 Detergent Solution is highly purified NP-40 detergent stabilized as a 10% solution in your choice of either 10mL glass ampules or two sizes of HDPE-plastic bottles.

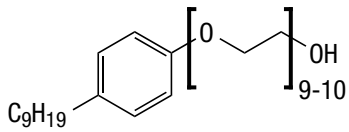
This easy-to-use 10% (w/v) solution of purified NP-40 detergent is ideal for use in routine and high-demand protein research methods and molecular biology techniques. NP-40 detergent is a nonionic polyoxyethylene surfactant that is most frequently used as a component of cell lysis buffers or other solutions intended to extract and solubilize proteins. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or non-leaching HDPE bottles, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

- **Accurate** – precise 10% detergent solutions in ultrapure water
- **Easy to use** – solutions are simple to dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged under inert nitrogen gas in glass ampules or HDPE bottles

Properties of NP-40 Detergent:

- Molecular Weight: 617g
- Detergent Class: Nonionic
- Aggregation Number: 149
- Micelle Molecular Weight: 90,000g
- Critical Micelle Concentration (CMC): 0.29mM (0.0179%, w/v)
- Cloud Point: 80°C
- Dialyzable: No



NP-40 Detergent
MW 617

Specifications for Surfact-Amps NP-40 Detergent Solution:

- Visual: Clear, colorless solution, free of particulate matter
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference

Table 1. Purity comparison of NP-40 detergents.

Manufacturer/Brand	Peroxide Concentration (ueq/mL)	Carbonyl Concentration (ueq/mL)
Thermo Scientific	≤ 0.035	≤ 0.01
Amresco	0.083	0.374
Anatrace	0.053	4.246
G-Bioscience	≤ 0.035	≤ 0.01
Millipore EMD	≤ 0.035	0.042
Roche	0.056	0.021

Ordering Information

Product #	Description	Pkg. Size
28324	**Surfact-Amps NP-40 Detergent Solution**	6 x 10mL
85124	**Surfact-Amps NP-40 Detergent Solution**	50mL
85125	**Surfact-Amps NP-40 Detergent Solution**	500mL

Thermo Scientific Surfact-Amps Brij-35 Detergent Solutions

Provides purity, quality and stability in convenient 10% and 30% solutions.

Thermo Scientific™ Surfact-Amps™ Brij™-35 Detergent Solutions are stabilized 10% and 30% solutions of Brij-35 detergent for use in various protein methods.

Brij-35 detergent is a nonionic polyoxyethylene surfactant that is most frequently used as a component of cell lysis buffers or a surfactant in various HPLC applications. The detergent is offered in two forms: a Surfact-Amps Detergent Solution (high-purity, 10% solution) and a standard grade 30% solution. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or HDPE bottles, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

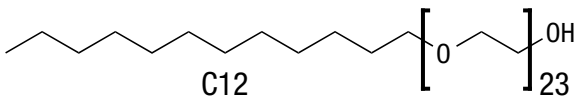
- **Accurate** – 10% and 30% detergent solutions in ultrapure water
- **Easy to use** – solutions are simple to dispense and dilute for use

Surfact-Amps 10% Detergent Solutions:

- **Accurate** – precise 10% detergent solution in ultrapure water
- **Easy to use** – solution is simple to dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged in under inert nitrogen gas in glass ampules or HDPE bottles

Properties of Brij-35 Detergent:

- Molecular Weight: 1225g
- Detergent Class: Nonionic
- Aggregation Number: 40
- Micelle Molecular Weight: 49,000g
- Critical Micelle Concentration (CMC): 0.09mM (0.011%, w/v)
- Cloud Point: > 100°C
- Dialyzable: No



Brij-35 Detergent
MW 1225

Specifications for Surfact-Amps Brij-35 Detergent Solution (10% solution):

- Visual: Clear, colorless solution, free of particulate matter
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference

Specifications for Brij-35, 30% Solution (Product # 20150):

- Visual: Clear, colorless viscous liquid, free of foreign material
- Concentration: Concentration: 27.0 to 30.0%

Table 1. Purity comparison of Brij-35 detergents.

Manufacturer/Brand	Peroxide Concentration (ueq/mL)	Carbonyl Concentration (ueq/mL)
Thermo Scientific	< 0.035	< 0.62
Amresco	1.075	3.742
Anatrace	< 0.035	< 0.62
G-Bioscience	< 0.035	< 0.62
Millipore EMD	< 0.035	< 0.62

Ordering Information

Product #	Description	Pkg. Size
28316	**Surfact-Amps Brij-35 Detergent Solution** Formulation: 10% (w/v) aqueous solution of Brij-35	6 x 10mL
85117	**Surfact-Amps Brij-35 Detergent Solution** Formulation: 10% (w/v) aqueous solution of Brij-35	50mL
85118	**Surfact-Amps Brij-35 Detergent Solution** Formulation: 10% (w/v) aqueous solution of Brij-35	500mL
20150	**Brij-35, 30% Solution** Formulation: 30% (w/v) aqueous solution of Brij-35	950mL

Thermo Scientific Surfact-Amps Brij-58 Detergent Solution

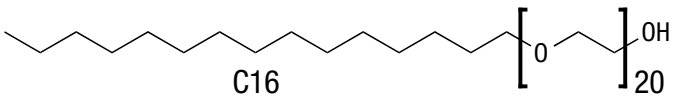
Purity, quality and stability in a convenient 10% solution.

Thermo Scientific™ Surfact-Amps™ Brij-58 Detergent Solution is highly purified Brij-58 detergent stabilized and supplied as a 10% solution in 10mL glass ampules.

This is an easy-to-use 10% (w/v) solution of purified Brij-58 detergent for use in routine and high-demand protein research methods and molecular biology techniques. Brij-58 detergent is a nonionic polyoxyethylene surfactant that is most frequently used in HPLC applications. Surfact-Amps Detergent Solutions (10% w/v) provide unsurpassed purity, quality and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% Solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules, ensuring their stability and eliminating the accumulation of peroxides and degradation products.

Highlights:

- **Accurate** – precise 10% detergent solution in ultrapure water
- **Easy to use** – simple to dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Stable** – packaged in glass ampules under inert nitrogen gas



Brij-58 Detergent
MW 1120

Properties of Brij-58 Detergent:

- Molecular Weight: 1120g
- Detergent Class: Nonionic
- Aggregation Number: 70
- Micelle Molecular Weight: 82,000g
- Critical Micelle Concentration (CMC): 0.077mM (0.0086%, w/v)
- Cloud Point: > 100°C
- Dialyzable: No

Specifications for Surfact-Amps Brij-58 Detergent Solution:

- Visual: Clear to slightly hazy, colorless solution, free of particulates
- Concentration: 10.0 ±1.0%
- Oxidants: ≤ 1.0µeq/mL
- Carbonyls: ≤ 1.0µeq/mL
- Suspended Solids: Residue present must not exceed Residue Reference

Ordering Information

Product #	Description	Pkg. Size
28336	**Surfact-Amps Brij-58 Detergent Solution**	6 x 10mL

Thermo Scientific Octyl-β-Glucoside

A nonionic detergent widely used for membrane protein solubilization.

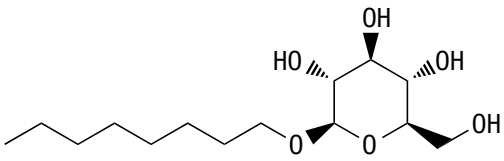
Thermo Scientific™ Octyl-β-Glucoside is a low-molecular weight, nonionic detergent that has been widely used for membrane protein solubilization.

Highlights:

- A low-molecular weight, nonionic detergent
- Effective for membrane protein solubilization
- Less stable and less commonly used than octylthioglucoside (OTG)
- Can be removed from solution by dialysis
- Optically clear; low absorbance at 280nm

Properties of N-Octyl-β-D-glucoside:

- Alternative Names: Octyl-beta-glucoside, OG, Octyl-beta-glucopyranoside, Octyl-beta-D-glucopyranoside
- Chemical Name: n-Octyl-β-D-glucoside
- Molecular Weight: 292.37g
- Detergent Class: Nonionic
- Aggregation Number: 27
- Micelle Molecular Weight: 8000g
- Critical Micelle Concentration (CMC): 23 to 25mM (0.6716 to 0.7300%, w/v)
- Cloud Point: >100°C
- Dialyzable: Yes



Octyl β-Glucoside
MW 292

References

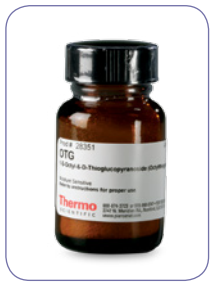
1. Rosevear, P., *et al.* (1980). *Biochemistry* **19:**4108-4115.
2. Gould, R.J., *et al.* (1981). *Biochemistry* **20:**6776-6781.
3. Jackson, M.L., *et al.* (1982). *Biochemistry* **21:**4576-4582.

Ordering Information

Product #	Description	Pkg. Size
28310	Octyl-β-Glucoside	5g

Thermo Scientific Octyl-β-thioglucoside

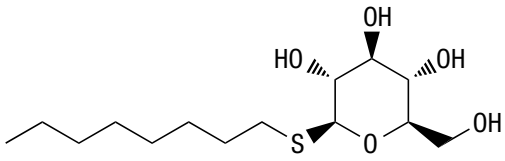
Stabilizes proteins better than octyl-β-glucoside.



Thermo Scientific™ Octyl-β-thioglucoside is a low-molecular weight, nonionic detergent that is effective for cell lysis and nondenaturing protein solubilization. It is resistant to β-D-glucoside glucohydrolase degradation.

Highlights:

- A low-molecular weight, nonionic detergent
- Effective for a variety of cell lysis and protein solubilization methods
- Unaffected by β-glucosidase enzyme
- More stable than octyl-β-glucoside (OG)
- Can be removed from solution by dialysis
- Optically clear; low absorbance at 280nm



Octylthioglucoside (OTG)
1-s-octyl-β-D-thioglucopyranoside
MW 308

Properties of n-Octyl-β-D-thioglucopyranoside:

- Alternative Names: Octylthioglucoside, OTG
- Chemical Name: n-Octyl-β-D-thioglucopyranoside
- Molecular Weight: 308.44g
- Detergent Class: Nonionic
- Aggregation Number: Unknown
- Micelle Molecular Weight: Unknown
- Critical Micelle Concentration (CMC): 9mM (0.2772%, w/v)
- Cloud Point: > 100°C
- Dialyzable: Yes

Specifications for Octylthioglucoside (OTG):

- Visual: White powder, free of foreign material
- Solubility: Solution must be clear, colorless and free of particulate matter
- Identity: The IR scan must show only peaks characteristic for the structure and functional groups of the compound being tested

References

1. Saito, S., *et al.*, (1984). *J. Biochem.* **222:**829-832.
2. Hanatani, M., *et al.*, (1989). *J. Biochem.* **95:**1349-1353.

Ordering Information

Product #	Description	Pkg. Size
28351	Octyl-β-thioglucoside (OTG)	5g

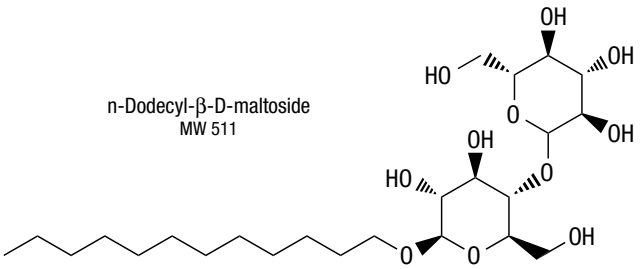
Thermo Scientific n-Dodecyl-β-D-Maltoside

Solubilizes membrane proteins and preserves activity.

Thermo Scientific™ n-Dodecyl-β-D-Maltoside is most often used for the isolation of hydrophobic membrane proteins. This detergent has dual hydrophobic/hydrophilic properties that facilitate lipid displacement and provide a lipid-like environment for membrane proteins. Studies suggest that the ability of these surfactants to preserve membrane protein structure stems in part from the reduced disruption of lipid:protein interactions where some of the natural lipid associations are maintained. This water-soluble nonionic detergent is most often used for the isolation of hydrophobic membrane proteins. Multiple studies have shown that n-Dodecyl-β-D-maltoside is a gentle detergent that is often able to preserve protein activity better than many commonly used detergents, including NP-40, CHAPS and Octyl-β-glucoside.

Highlights:

- Lipid-like nonionic detergent
- Especially useful for isolating and stabilizing hydrophobic membrane proteins
- Preserves activity of membrane protein better than most of the detergents
- High-purity compound with low UV absorptivity



Properties of n-Dodecyl-β-D-Maltoside:

- Chemical Name: n-Dodecyl-β-D-maltoside
- Molecular Weight: 510.6g
- Detergent Class: Nonionic
- Aggregation Number: 98 (average), 70 to 140 range
- Micelle Molecular Weight: 50,000g
- Critical Micelle Concentration (CMC): 0.17mM (0.009%, w/v) in water; 0.12mM (0.006%, w/v) in 0.2M NaCl
- Cloud Point: Unknown
- Dialyzable: No

Ordering Information

Product #	Description	Pkg. Size
89902	n-Dodecyl-β-D-maltoside, > 99% Purity	1g
89903	n-Dodecyl-β-D-maltoside, > 99% Purity	5g

Thermo Scientific Surfact-Amps Detergent Sampler

Convenient 10-sample package of detergents allows for trial testing and experimentation.

The Thermo Scientific™ Surfact-Amps™ Detergent Sampler is a convenient collection of various Surfact-Amps Detergent Solutions and selected other useful detergents for protein research methods. The sampler kit is an economical way to test which detergent is most effective for a particular protein storage or experimental system.

Surfact-Amps Purified Detergent Solutions are convenient 10% (w/v) solutions of commonly used detergents that provide unsurpassed purity, quality and stability for proteomics and molecular biology methods. Unlike neat detergent formulations, Surfact-Amps 10% Solutions are not so viscous that you can't aliquot them accurately. The detergent solutions are carefully prepared and packaged under nitrogen in glass ampules, ensuring their stability and eliminating the accumulation of peroxides and degradation products. Three of the 10 detergents in the sampler kit are supplied as solids (100mg each) rather than 10% solutions.

Highlights:

- **Accurate** – precise 10% detergent solutions in ultrapure water
- **Easy to use** – easy to accurately dispense and dilute for use
- **Exceptionally pure** – less than 1.0µeq/mL peroxides and carbonyls
- **Highly stable** – packaged in glass ampules under inert nitrogen gas

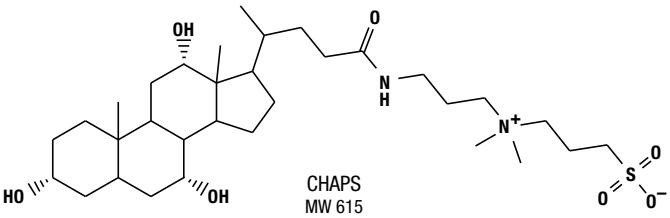
Ordering Information

Product #	Description	Pkg. Size
28340	Surfact-Amps Detergent Sampler	Kit
	Includes: Surfact-Amps Purified Detergents	
	Surfact-Amps Triton X-100 (10%)	10mL
	Surfact-Amps Brij-35 (10%)	10mL
	Surfact-Amps Tween-20 (10%)	10mL
	Surfact-Amps NP-40 (10%)	10mL
	Surfact-Amps Tween-80 (10%)	10mL
	Surfact-Amps Triton X-114 (10%)	10mL
	Surfact-Amps Brij-58 (10%)	10mL
	Octyl β-Glucoside	100mg
	Octyl β-Thioglucopyranoside	100mg
	CHAPS	100mg

Thermo Scientific CHAPS Detergent

Zwitterionic detergent that protects the native state of proteins.

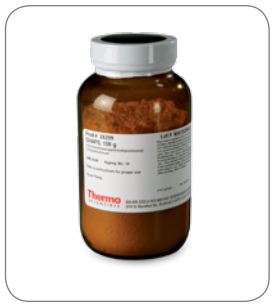
Thermo Scientific™ CHAPS Detergent is a zwitterionic detergent that is especially well suited for protecting the native state of proteins. CHAPS is sulfobetaine derivative of cholic acid, and is useful for membrane protein solubilization when it is important to maintain protein activity. CHAPS has been successfully used to solubilize intrinsic membrane proteins and receptors and maintain the functional capability of the protein of interest. CHAPS has also been used in combination with nonionic detergents such as NP-40 for nondenaturing gel electrophoresis applications. CHAPS has largely replaced NP-40 in isoelectric focusing (IEF) applications, where it prevents streaking in certain pH ranges. The neutral charge of CHAPS and its disaggregating properties are believed to be responsible for the improvement. CHAPS detergent is soluble over a wide range of pH (2 to 12) and is easily removed from solution by dialysis because it has a high critical micelle concentration (CMC).



CHAPS
MW 615

Highlights:

- Versatile zwitterionic detergent
- Provides mild but effective lysis of cultured mammalian cells
- Nondenaturing and generally does not inactivate protein functions
- Can be removed from solution by dialysis
- Commonly used for isoelectric focusing (IEF) and 2-D electrophoresis



Properties of CHAPS Detergent:

- Chemical Name: 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate
- Molecular Weight: 614.88g
- Detergent Class: Zwitterionic
- Aggregation Number: 10
- Micelle Molecular Weight: 6149g
- Critical Micelle Concentration (CMC): 8 to 10mM (0.4920 to 0.6150%, w/v)
- Cloud Point: ≥ 100°C
- Dialyzable: Yes

References

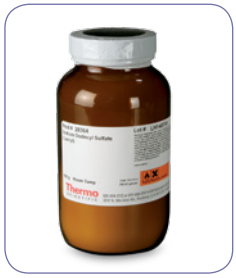
1. Simonds, W.F., *et al.* (1980). *Proc. Natl. Acad. Sci.* (USA) **77:**4623-4627.
2. Evans, E.A., *et al.* (1986). *Proc. Natl. Acad. Sci.* (USA) **83:**581-585.
3. Kuno, T., *et al.* (1984). *J. Neurochem.* **41:**841.
4. Kuno, T., *et al.* (1983). *BBRC* **112:**948.
5. Chow, T., *et al.* (1983). *Mol. Pharmacol.* **24:**203.

Ordering Information

Product #	Description	Pkg. Size
28300	**CHAPS** (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate)	5g
28299	**CHAPS**	100g

Thermo Scientific Sodium Dodecyl Sulfate (Lauryl SDS)

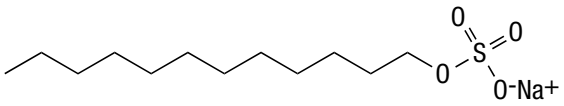
Electrophoresis- and lysis buffer-grade SDS formulation.



Thermo Scientific™ Sodium Dodecyl Sulfate (Lauryl) is standard-grade SDS detergent for use in protein polyacrylamide gel electrophoresis (PAGE). This lauryl-grade sodium dodecyl sulfate (SDS) is a popular anionic detergent for routine protein electrophoresis and cell lysis methods. The formulation is a mixture of several different alkyl sulfate chain lengths (C_{10} to C_{18}).

Highlights:

- Popular anionic detergent for a variety of protein methods
- Especially useful for denaturing polyacrylamide gel electrophoresis (SDS-PAGE)
- Common component of cell lysis buffer



Sodium dodecyl sulfate (SDS)
MW 288

Properties of SDS (values for pure C_{12}):

- Molecular Weight: 288.5g
- Detergent Class: Ionic (anionic)
- Aggregation Number: 62
- Micelle Molecular Weight: 18,000g
- Critical Micelle Concentration (CMC): 6 to 8mM (0.1728 to 0.2304%, w/v)
- Cloud Point: > 100°C
- Dialyzable: No

Ordering Information

Product #	Description	Pkg. Size
28364	SDS, Lauryl	100g
28365	SDS, Lauryl	1kg

Thermo Scientific Sodium Dodecyl Sulfate (SDS) C_{12}

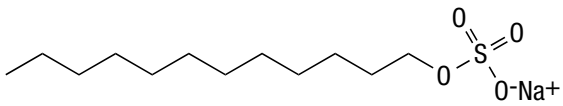
Highly purified SDS formulation with 98% of the C_{12} alkyl sulfate.

Thermo Scientific™ Sodium Dodecyl Sulfate (C_{12}) is a highly purified form of SDS that is especially suited for protein experiments requiring tightly controlled solubility parameters.

Sodium dodecyl sulfate (SDS) is an alkyl sulfate, anionic detergent used frequently in protein electrophoresis and protein solubilization methods. It is a component of many cell lysis buffer formulations, including RIPA buffer. Typical SDS or lauryl sulfate preparations are mixtures of several different chain lengths (C_{10} to C_{18}). However, protein renaturation and other specialized methods require very specific surfactant and buffer conditions. This particular formulation of SDS is highly purified to eliminate nearly all secondary chain lengths besides C_{12}.

Highlights:

- Popular anionic detergent for protein electrophoresis and solubilization
- Component of many cell lysis buffer formulations
- Specially purified formulation is > 98% C_{12} alkyl sulfate
- Contains low levels of hexadecyl sulfate (C_{16}), which inhibits protein renaturation



Sodium dodecyl sulfate (SDS)
MW 288

Properties of Sodium Dodecyl Sulfate, (pure C_{12}):

- Molecular Weight: 288.5g
- Detergent Class: Ionic (anionic)
- Aggregation Number: 62
- Micelle Molecular Weight: 18,000g
- Critical Micelle Concentration (CMC): 6 to 8mM (0.1728 to 0.2304%, w/v)
- Cloud Point: > 100°C
- Dialyzable: No

Ordering Information

Product #	Description	Pkg. Size
28312	SDS, C_{12} (Sodium dodecyl sulfate, C_{12})	500g

Thermo Scientific Pierce Detergent Removal Products

Optimized detergent removal for samples with protein or peptide concentrations > 100µg/mL.

Thermo Scientific™ Pierce™ Detergent Removal Resin works with high concentrations of a broad range of commonly used detergents, while providing exceptional results.

The Thermo Scientific™ Pierce™ Detergent Removal Spin Plates provide a high-throughput method for effectively removing detergents from samples. Thermo Scientific™ Pierce™ Detergent Removal Spin Columns provide flexibility and are available in a variety of sizes to treat both protein and peptide samples ranging from 0.01mL to 1mL.

Highlights:

- **High Performance** – removes detergent with > 90% recovery and no sample dilution
- **Versatile** – effectively removes detergents from both peptide or protein samples
- **Optimized** – improves MS peptide coverage
- **Convenient** – available in various formats, including 96-well spin plates, spin columns and bulk resin
- **Fast and efficient protocol** – samples can be processed in 15 minutes

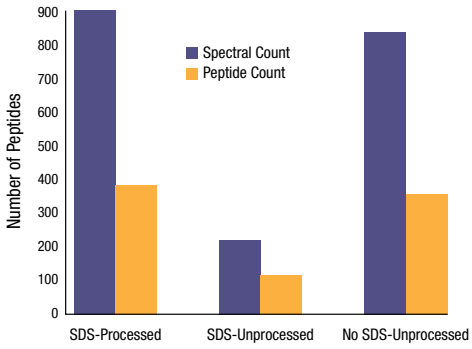
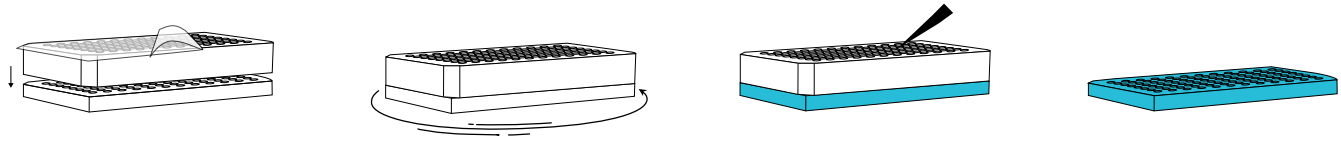


Figure 1. Effective detergent removal enables greater peptide identification. A tryptic digest of HeLa cell lysate (0.1mL, 100µg) containing 1% SDS was processed through 0.5mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. The processed sample allowed similar numbers of identified peptides as digests containing no SDS. Peptide identification is greatly reduced in sample containing SDS.



1. Remove the bottom seal and stack the detergent removal plate on top of a wash plate. Remove the top seal and centrifuge.†

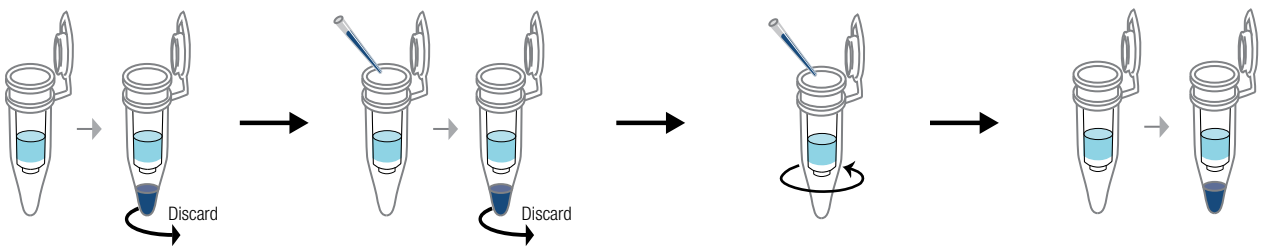
2. Add 300µL of buffer to each well and centrifuge. Discard the flow-through. Repeat this step two times.

3. Stack the detergent removal plate on top of a sample collection plate. Apply sample and incubate at room temperature for 2 minutes. Centrifuge to remove detergent.

4. Recover the detergent-free sample for downstream analysis.

† Centrifugations are performed for 2 minutes at 1000 x *g*.

Figure 2. Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Plates.



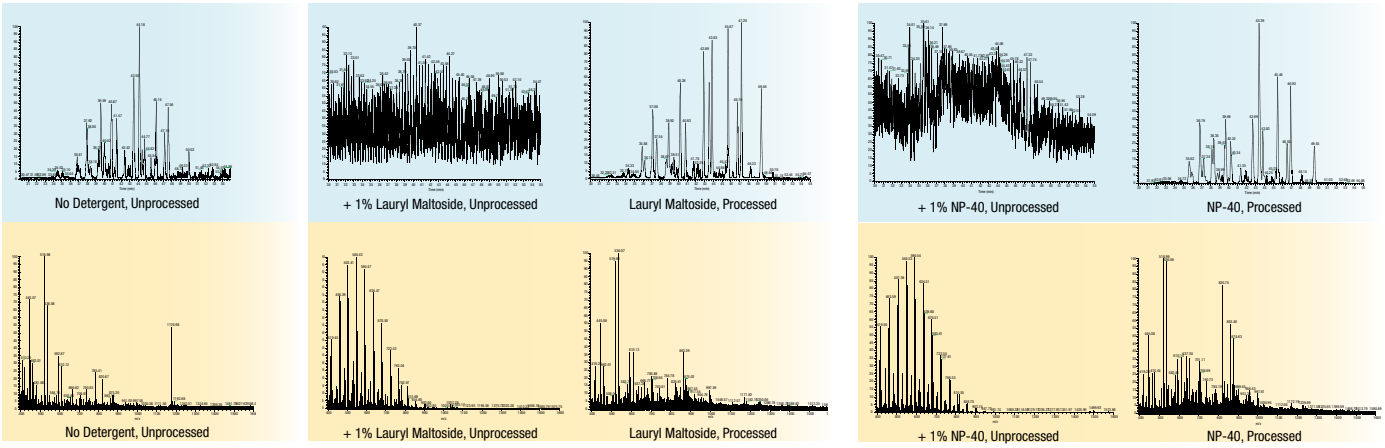
1. Centrifuge for 1 minute at 1500 x *g* to remove the storage buffer.

2. Add 0.4mL equilibration buffer, centrifuge at 1500 x *g* for 1 minute and discard the flow-through. Repeat two additional times.

3. Add detergent-containing sample (25-100µL) and incubate for 2 minutes at RT.

4. Centrifuge at 1500 x *g* for 2 minutes to collect the detergent-free sample for downstream applications.

Figure 3. Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Columns (0.5mL).



Base Peak LC-MS Chromatograms

Integrated Mass Spectra

Figure 4. Effective detergent removal eliminates interference and allows high sequence coverage analysis of BSA. Tryptic digests (0.1mL, 100µg) containing detergent were each processed through 0.5mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. **Top row:** Base peak LC-MS chromatograms. **Bottom row:** Integrated mass spectra. Similar results were produced for Brij-35 Detergent, octyl glucoside, octyl thioglucoside and SDS (data not shown).

Table 1. Effectiveness and protein recovery of detergent-removal resins.

Process Format†	Detergent	Detergent Concentration (%)	Detergent Removal (%)	BSA Recovery (%)
96-well Spin Plate	SDS	5	99	89
	Triton X-100	4	99	100
	NP-40	1	95	100
	CHAPS	5	99	100
0.5mL Spin Column	Sodium deoxycholate	5	99	100
	Octyl glucoside	5	99	90
	Octyl thioglucoside	5	99	95
	Lauryl maltoside	1	98	99
	Triton X-114	2	95	100
	Brij-35	1	99	97
	Tween-20	0.25	99	87

† *Each plate well and column contained ~550µL of detergent-removal resin slurry and 0.1mL of sample. Similar results were obtained with both process formats.*

Ordering Information

Product #	Description	Pkg. Size
88304	**Pierce 96-well Detergent Removal Spin Plates** *Sufficient for 25 to 100µL samples per well.*	2 plates
87776	**Pierce Detergent Removal Spin Column, 125µL** *Sufficient for 10 to 25µL sample per column.*	25 columns
87777	**Pierce Detergent Removal Spin Column, 0.5mL** *Sufficient for 25 to 100µL sample per column.*	25 columns
87778	**Pierce Detergent Removal Spin Column, 2mL** *Sufficient for 150 to 500µL sample per column.*	5 columns
87779	**Pierce Detergent Removal Spin Column, 4mL** *Sufficient for 500 to 1000µL sample per column.*	5 columns
87780	**Pierce Detergent Removal Resin**	10mL

Thermo Scientific HiPPR (High Protein and Peptide Recovery) Detergent Removal Columns and Plates

Optimized detergent removal for samples with protein or peptide concentrations between 1-100µg/mL.

Thermo Scientific™ HiPPR Detergent Removal Resin in spin column or 96-well filter plate formats improves MS results by efficiently removing detergents from 25 to 200µL samples with low protein or peptide concentrations.

The HiPPR (High Protein and Peptide Recovery) Detergent Removal Resin removes > 95% of detergents with minimal sample loss. The resin is ideal for removing commonly used detergents, including SDS, Triton X-100, NP-40 and CHAPS detergents at concentrations of 0.5-1% and is available in pre-filled spin columns and 96-well filter spin plates for sample volumes up to 100µL. The spin format has been optimized for protein or peptide concentrations from 1-100µg/mL. For other sample sizes, the detergent removal resin slurry is available with empty spin columns that can be used to make custom spin columns for processing sample volumes of 25 to 200µL.

Highlights:

- **Optimized** – removes > 95% of detergent from samples with low-concentrations (1 to 100µg/mL) of proteins or peptides
- **Fast** – sample processing takes less than 15 minutes
- **Effective** – eliminates detergent interference in downstream applications like ELISA, isoelectric focusing and MS

The HiPPR Detergent Removal Resin is ideal for rapid detergent removal from tryptic digests with low peptide concentrations and can help to improve the results of LC-MS/MS and MALDI-MS analysis, as well as to maintain column and instrument performance over time.

Table 1. Detergent removal efficiency and protein recovery.
BSA sample (25-200µL) + detergent in 0.15M NaCl, 0.05% sodium azide was mixed with equal volume of detergent removal resin (2X volume for CHAPS removal) and processed as shown in the protocol.

Detergent	Sample Volume (µL)	Protein Quantity (µg)	Detergent Removal (%)	Protein Recovery (%)
SDS (1%)	25	0.375	>99	98
	50	0.75	>99	97
	100	1.5	>99	100
	200	3.0	>99	100
Triton X-100 (1%)	25	0.375	>95	82
	50	0.75	>95	86
	100	1.5	>95	86
	200	3.0	>95	93
NP-40 (0.75%)	25	0.375	95	90
	50	0.75	96	94
	100	1.5	97	91
	200	3.0	97	97
CHAPS (1%)	25	0.375	95	64
	50	0.75	97	70
	100	1.5	98	78
	200	3.0	98	75

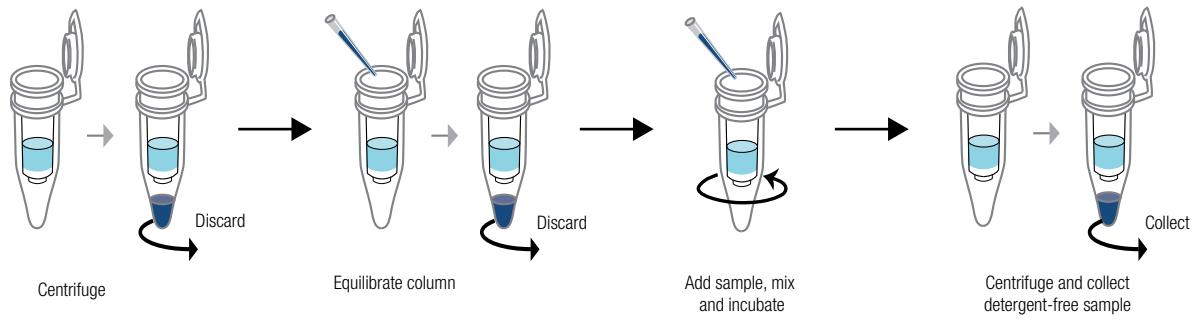


Figure 1. Protocol summary for Thermo Scientific HiPPR Detergent Removal Spin Columns.

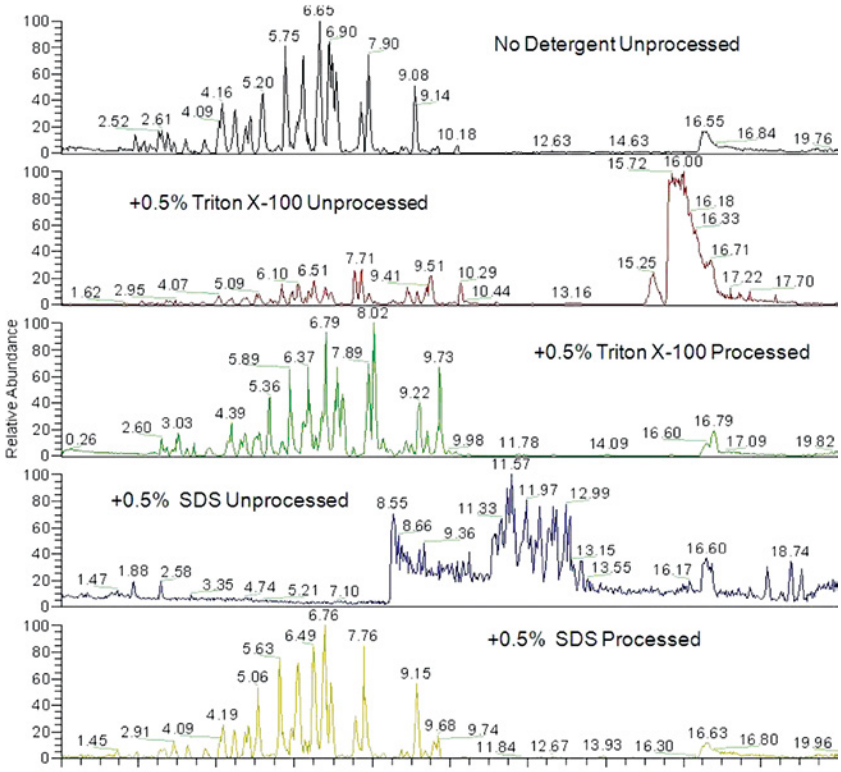


Figure 2. Thermo Scientific HiPPR Detergent Removal Resin improves LC-MS/MS analysis of enzymatically digested BSA. BSA (100µg/mL) tryptic digests were prepared without detergent, in the presence of 0.5% Triton X-100 or spiked with 0.5% SDS following enzymatic digestion. Samples (0.1mL) containing detergent were processed with the HiPPR Detergent Removal Resin and compared to unprocessed or detergent-free samples by LC-MS/MS. Results demonstrate that detergent removal is effective and produces results similar to those observed for samples containing no detergent.

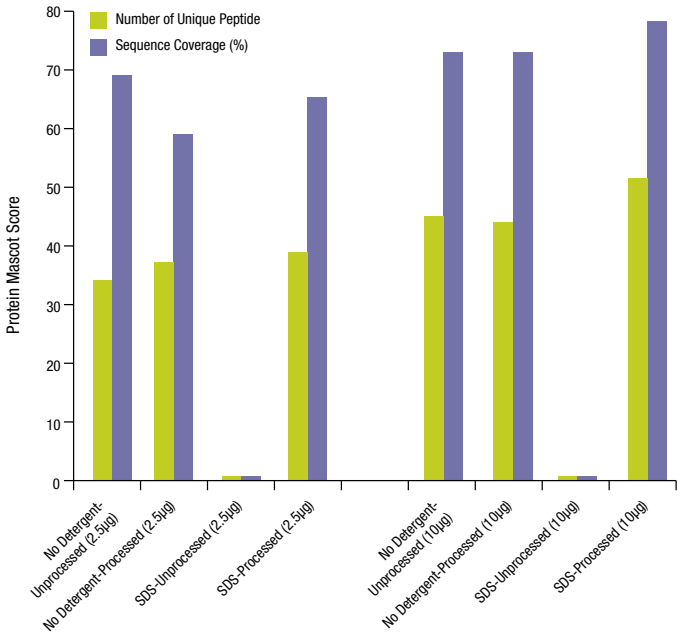


Figure 3. Effective detergent removal enables greater peptide identification. BSA (25 and 100µg/mL) was digested in the presence and absence of detergents and the samples were processed for LC-MS/MS analysis. Effective detergent removal resulted in greater peptide identification and high MASCOT scores.

Ordering Information

Product #	Description	Pkg. Size
88305	**HiPPR Detergent Removal Spin Column Kit**	5mL kit
	Includes: Detergent Removal Resin	5mL
	Spin Columns Accessory Pack	54 columns
88306	**HiPPR Detergent Removal Spin Columns, 0.1mL**	24
	Sufficient for 24 samples of 100µL each.	columns
88307	**HiPPR Detergent Removal 96-well Spin Plates, 0.1mL**	2 plates
	Sufficient for 192 samples of 100µL each.	
	Includes: HiPPR Detergent Removal 96-well Filter Spin Plate	2 plates
	96-well Deep-well Collection Plate	2 plates
	96-well Collection Plate	2 plates

Dialysis Products

Product #	Capacity	Pkg. Size
Slide-A-Lyzer MINI Dialysis Device, 2K MWCO		
69580	0.1mL	50 devices
69553	0.1mL	250 devices
Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO		
69550	0.1mL	50 devices
69552	0.1mL	250 devices
88400	0.5mL	25 devices
88403	2mL	25 devices
Slide-A-Lyzer MINI Dialysis Device, 7K MWCO		
69560	0.1mL	50 devices
69562	0.1mL	250 devices
Slide-A-Lyzer MINI Dialysis Device, 10K MWCO		
69570	0.1mL	50 devices
69572	0.1mL	250 devices
88401	0.5mL	25 devices
88404	2mL	25 devices
Slide-A-Lyzer MINI Dialysis Device, 20K MWCO		
69590	0.1mL	50 devices
69555	0.1mL	250 devices
88402	0.5mL	25 devices
88405	2mL	25 devices
Slide-A-Lyzer MINI Dialysis Device Floats		
69588	25-unit	4 floats
Slide-A-Lyzer G2 Dialysis Cassettes and Flasks, 2K MWCO		
87717	0.5mL	10 cassettes
87718	3mL	10 cassettes
87719	15mL	8 cassettes
87720	30mL	6 cassettes
87721	70mL	6 cassettes
87760	250mL	4 flasks

Product #	Capacity	Pkg. Size
Slide-A-Lyzer G2 Dialysis Cassettes and Flasks, 3.5K MWCO		
87722	0.5mL	10 cassettes
87723	3mL	10 cassettes
87724	15mL	8 cassettes
87725	30mL	6 cassettes
87726	70mL	6 cassettes
87761	230mL	4 flasks
Slide-A-Lyzer G2 Dialysis Cassettes, 7K MWCO		
87727	0.5mL	10 cassettes
87728	3mL	10 cassettes
Slide-A-Lyzer G2 Dialysis Cassettes and Flasks, 10K MWCO		
87729	0.5mL	10 cassettes
87730	3mL	10 cassettes
87731	15mL	8 cassettes
87732	30mL	6 cassettes
87733	70mL	6 cassettes
87762	250mL	4 flasks

Sterile Gamma-irradiated Slide-A-Lyzer G2 Dialysis Cassettes: check website

Slide-A-Lyzer G2 Dialysis Cassettes and Flasks, 20K MWCO		
87734	0.5mL	10 cassettes
87735	3mL	10 cassettes
87736	15mL	8 cassettes
87737	30mL	6 cassettes
87738	70mL	6 cassettes
87763	250mL	4 flasks
Slide-A-Lyzer Flotation Disk		
87759	-	6 disks

Desalting Products

Product #	Capacity	Pkg. Size
Zeba Spin Desalting Columns, 7K MWCO		
89877	75µL	25 columns
89878	75µL	50 columns
89882	0.5mL	25 columns
89883	0.5mL	50 columns
89889	2mL	5 columns
89890	2mL	25 columns
89891	5mL	5 columns
89892	5mL	25 columns
89893	10mL	5 columns
89894	10mL	25 columns
Zeba 96-well Spin Desalting Plates, 7K MWCO		
89807	-	2 plates
89808	-	4 plates
Zeba Desalting Chromatography Cartridges, 7K MWCO		
89934	1mL	5 cartridges
89935	5mL	5 cartridges

Product #	Capacity	Pkg. Size
Zeba Spin Desalting Columns, 40K MWCO		
87764	75µL	25 columns
87765	75µL	50 columns
87766	0.5mL	25 columns
87767	0.5mL	50 columns
87768	2mL	5 columns
87769	2mL	25 columns
87770	5mL	5 columns
87771	5mL	25 columns
87772	10mL	5 columns
87773	10mL	25 columns
Zeba 96-well Spin Desalting Plates, 40K MWCO		
87774	-	2 plates
87775	-	4 plates

Protein Concentrators

Product #	Capacity	Pkg. Size
Pierce Protein Concentrators PES		
88512	**3K MWCO**, 0.5mL	25 units
88513	**10K MWCO**, 0.5mL	25 units
88502	**30K MWCO**, 0.5mL	25 units
88503	**100K MWCO**, 0.5mL	25 units

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