INSTRUCTIONS

Subcellular Protein Fractionation Kit for Tissues

Pub. No. MAN0011756
Pub. Part No. 2318.1
Rev B.0

87790

Number	Description
87790	**Subcellular Protein Fractionation Kit for Tissues**, sufficient reagents for 25 fractionations from 200mg of tissue (a total of 5g of tissue)
	Kit Contents:
	Cytoplasmic Extraction Buffer (CEB) for Tissues, 50mL, store at -20°C
	Membrane Extraction Buffer (MEB) for Tissues, 35mL, store at 4°C
	Nuclear Extraction Buffer (NEB) for Tissues, 20mL, store at 4°C
	Pellet Extraction Buffer (PEB) for Tissues, 6.5mL, store at room temperature
	Micrococcal Nuclease, ≥ 100 units/µL, 260µL, store at -20°C
	Calcium Chloride ($CaCl_2$), 100mM, 450µL, store at 4°C
	Halt Protease Inhibitor Cocktail, 100X, 1mL, store at 4°C
	Pierce Tissue Strainer, 250µm, 25 devices, store at room temperature
	Storage: Upon receipt store kit at -20°C or store individual components as indicated above. Kit is shipped with dry ice.

Introduction

The Thermo Scientific™ Subcellular Protein Fractionation Kit for Tissues enables stepwise separation and preparation of cytoplasmic, membrane, nuclear soluble, chromatin-bound and cytoskeletal protein extracts from tissue samples. The kit includes four high-performance extraction buffers. The first extraction buffer selectively permeabilizes the cell membrane, releasing soluble cytoplasmic contents. A Thermo Scientific™ Pierce™ Tissue Strainer removes tissue debris before the second buffer dissolves plasma, mitochondria and ER/golgi membranes without solubilizing the nuclear membranes. After recovering the intact nuclei by centrifugation, a third buffer yields the soluble nuclear extract. A second nuclear extraction with micrococcal nuclease is performed to release chromatin-bound nuclear proteins. The recovered insoluble pellet is then extracted with the final buffer to isolate cytoskeletal proteins.

Extracts obtained with this kit are compatible with a variety of downstream applications, including Western blotting, the Thermo Scientific™ Pierce™ BCA Protein Assay (Product No. 23225), the Thermo Scientific™ LightShift™ Chemiluminescent EMSA Kit (Product No. 20148) and reporter-gene and enzyme-activity assays. Extracts from each subcellular compartment generally have less than 15% contamination between fractions, which is sufficient purity for most experiments studying protein localization and redistribution.

Important Procedural Notes

- For best results, use fresh tissue. If the tissue sample must be frozen, ensure it is snap frozen and stored at -80°C.
- For optimal results, use 200mg of tissue per extraction. Although less than 200mg of tissue may be used, using less than 50mg of tissue is not recommended because of the small homogenization volume.
- Thaw all buffers using a water bath at room temperature; keep CEB, MEB and NEB on ice until use. If precipitate occurs in PEB, mix vigorously to resuspend; however, the presence of a precipitate does not adversely affect PEB performance.
- Protease inhibitors are required to maintain extract integrity and function. Immediately before use, add protease inhibitors to CEB, MEB, NEB and PEB by diluting Thermo Scientific™ Halt™ Protease Inhibitor Cocktail 1:100 into each volume of buffer required.

- Perform all incubations and centrifugations at 4°C unless otherwise noted. Keep cell samples and extracts on ice unless otherwise noted.
- Use a rotary shaker to avoid clumping of insoluble material during incubations.
- Subcellular protein extracts can be used directly in many downstream assays. Some applications might require dialysis or desalting to remove detergent and salts. Although the detergent in the MEB is not dialyzable, it does not interfere with isoelectric focusing. PEB contains a strong denaturing detergent that is not compatible with isoelectric focusing. For 2D analysis of cytoskeletal proteins, resuspend the pellet directly in 2D sample buffer.

Additional Materials Required

- Ice-cold phosphate-buffered saline (PBS): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Thermo Scientific™ BupH™ Phosphate Buffered Saline Packs, Product No. 28372)
- Tissue grinder (e.g., a Dounce tissue homogenizer or Polytron™ handheld homogenizer)
- Rotary shaker in a cold room
- Centrifuge capable of 4°C and holding 15mL conical tubes
- Microcentrifuge

Tissue Preparation

1. Weigh 50-200mg of tissue, wash gently with ice-cold PBS and blot carefully on a lab wipe to remove excess liquid.
2. Cut tissue into small pieces and place in an appropriate pre-chilled homogenization tube. Use either a Dounce tissue homogenizer or a tube large enough for a Polytron handheld homogenizer.
3. Add ice-cold CEB containing protease inhibitors to the tissue (see Table 1) and homogenize. Proceed to the Subcellular Protein Fractionation Section and use the reagents as indicated in Table 1.

   Note: Homogenize for ~10-20 strokes with a Dounce tissue homogenizer or ~10-20 seconds with a Polytron handheld homogenizer on Speed 2. The amount of homogenization required will depend on the hardness of the tissue. For example, brain tissue will require less homogenization than heart tissue.

Table 1. Reagent volumes for different tissue amounts.

Tissue weight (mg)	CEB (µL)	MEB (µL)	NEB (µL)	NEB (µL) +$CaCl_2$, MNnase*	PEB (µL)
50	500	325	110	80	60
100	1000	650	225	170	125
200	2000	1300	450	340	250

*MNase = Micrococcal Nuclease

Subcellular Protein Fractionation

Note: Scale this protocol depending on the tissue weight (Table 1). Maintain the volume ratio of CEB:MEB:NEB:PEB reagents at 2000:1300:340:250µL, respectively.

1. Place a Pierce Tissue Strainer into a 15mL conical tube on ice.
2. Transfer the homogenized tissue into the Pierce Tissue Strainer. Centrifuge the strainer in the tube at 500 × *g* for 5 minutes. Immediately transfer the supernatant (cytoplasmic extract) into a clean pre-chilled tube on ice.
3. Add ice-cold MEB containing protease inhibitors to the pellet. Vortex the tube for 5 seconds on the highest setting and incubate the tube at 4°C for 10 minutes with gentle mixing.
4. Centrifuge at 3000 × *g* for 5 minutes.
5. Transfer the supernatant (membrane extract) into a clean pre-chilled tube on ice.
6. Add ice-cold NEB containing protease inhibitors to the pellet. Vortex the tube on the highest setting for 15 seconds and incubate the tube at 4°C for 30 minutes with gentle mixing.

7. Centrifuge the tube at 5000 × *g* for 5 minutes. Transfer the supernatant (soluble nuclear extract) fraction into a clean pre-chilled tube on ice.
8. Prepare the chromatin-bound extraction buffer by adding 5µL of 100mM $CaCl_2$ and 3µL of Micrococcal Nuclease (300 units) per 100µL of room-temperature NEB.
9. Add room-temperature NEB containing protease inhibitors, $CaCl_2$ and Micrococcal Nuclease to the pellet. Vortex on the highest setting for 15 seconds.
10. Incubate the tube at room temperature for 30 minutes or in a 37°C water bath for 15 minutes.
11. Vortex the tube on the highest setting for 15 seconds and centrifuge at 16,000 × *g* (highest microcentrifuge setting) for 5 minutes.
12. Transfer the supernatant (chromatin-bound nuclear extract) fraction into a clean pre-chilled tube on ice.
13. Add room-temperature PEB containing protease inhibitors to the pellet. Vortex on the highest setting for 15 seconds and incubate at room temperature for 10 minutes.
14. Centrifuge the tube at 16,000 × *g* (i.e., the highest microcentrifuge setting) for 5 minutes. Transfer the supernatant (i.e., the cytoskeletal extract) into a new tube.

    Note: For same-day use, maintain fractions on ice for downstream applications and analysis. For long-term storage, store fractions at -80°C.

Troubleshooting

Problem	Possible Cause	Solution
Low cytoplasmic protein yield	Tissue disruption was too gentle	Increase homogenization time or strokes
	CEB was stored improperly	Store CEB at -20°C
Low membrane protein yield	Membranes solubilized with excessive homogenization in CEB	Decrease homogenization time or strokes
	Incomplete membrane protein isolation	Increase time in MEB
Low soluble nuclear protein yield	Nuclei were not extracted	Vortex thoroughly
	Incomplete isolation of nuclei	Increase time of centrifugation after adding MEB
Low chromatin-bound protein yield	Calcium chloride or micrococcal nuclease was not added	Add $CaCl_2$ and micrococcal nuclease to NEB before extraction
	Micrococcal nuclease was stored improperly	Store micrococcal nuclease at -20°C
	Chromatin was not completely degraded	Vortex thoroughly
		Add more micrococcal nuclease
		Incubate longer at 37°C
Low overall protein yield	Volumes of extraction reagents were not appropriate for the given tissue weight	Use the reagent volumes listed in Table 1

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Proteins are not compartmentalized	Incomplete lysis	Remove all PBS before adding CEB
		Increase homogenization time or strokes
		Vortex longer to completely disperse the pellet
		Increase incubation time at each step
	Incomplete removal of extracts	Carefully remove all extract before proceeding to the next step
		Re-centrifuge sample and remove excess extract
		Rinse pellets with additional extraction buffers or PBS

Related Thermo Scientific Products

28348 **20X Phosphate Buffered Saline,** 500mL

87785 **Halt Protease Inhibitor Cocktail, EDTA-free (100X)**

87786 **Halt Protease Inhibitor Cocktail (100X)**

78420 **Halt Phosphatase Inhibitor Cocktail,** 1mL

88216 **Micrococcal Nuclease, ≥ 100 units/µL,** 150µL

78833 **NE-PER™ Nuclear and Cytoplasmic Extraction Reagents,** 1 kit

87792 **N-PER™ Neuronal Protein Extraction Reagent,** 100mL

20148 **LightShift Chemiluminescent EMSA Kit**

23224 **Pierce BCA Protein Assay Reagent Kit**

22660 **Pierce 660nm Protein Assay Reagent**

87791 **Pierce Tissue Strainers, 250µm,** 25/pkg

88400 **Slide-A-Lyzer™ MINI Dialysis Device, 3.5K MWCO, 0.5mL,** 25/pkg

88403 **Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 2mL,** 25/pkg

78840 **Subcellular Protein Fractionation Kit**

87793 **Syn-PER™ Synaptic Protein Extraction Reagent,** 100mL

89882 **Zeba™ Spin Desalting Columns, 0.5mL,** 25/pack

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | thermofisher.com