INSTRUCTIONS

Pierce™ 660nm Protein Assay

MAN0016386
Rev. B.0
Pub. Part No. 2162070

22660 22662

Number	Description
22660	**Pierce 660nm Protein Assay Reagent,** 750mL, contains sufficient reagents for 500 test tube or 5000 microplate assays
22662	**Pierce 660nm Protein Assay Kit,** sufficient reagents for 300 test tube or 3000 microplate assays **Kit Contents:** **Pierce 660nm Protein Assay Reagent,** 450mL **Pre-diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set,** 7 × 3.5mL, contains standardized BSA solutions at a specific concentration from 125 to 2000µg/mL in 0.9% saline and 0.05% sodium azide

Storage: Upon receipt store the assay reagent at room temperature and the pre-diluted BSA standards at 4ºC. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Pierce™ 660nm Protein Assay is a quick, ready-to-use colorimetric method for total protein quantitation. The assay is reproducible, rapid and more linear compared to coomassie-based Bradford assays and compatible with high concentrations of most detergents, reducing agents and other commonly used reagents. The assay has a moderate protein-to-protein variation.

This simple assay is performed in either test tube or a microplate. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions assayed alongside the unknown samples. The best relative standard to use gives a color response similar to that of the protein being assayed. The two most common protein standards for protein assays are BSA and BGG. The BSA standard is an appropriate standard if the sample contains primarily albumin. The BGG standard is an appropriate standard if the sample contains primarily globulins.

Important Product Information

- Certain substances interfere with the Pierce 660nm Protein Assay. Please see the Interfering Substances section for more information.
- For best results, protect the Pierce 660nm Protein Assay Reagent from the external environment by limiting exposure to the air and ensuring the cap is secure between uses.
- Precipitate may form in the Pierce 660nm Protein Assay Reagent over time. This may be more noticeable if the product is exposed to air for extended periods of time. Minimal precipitation will not impact performance and can be removed by centrifugation.

Procedure for the Pierce 660nm Protein Assay

Sample Preparation

- For samples containing >0.0125% SDS, add one pack of Ionic Detergent Compatibility Reagent (IDCR, Product No. 22663) to 20mL of the Pierce 660nm Protein Assay Reagent before performing the assay. The IDCR Solution is stable for 24 hours at room temperature. Mix the solution before each use.
- For cells lysed in Laemmli sample buffer, dilute the lysate from 1:10 to 1:20 in Laemmli buffer. Also add one pack of IDCR to 20mL of the Pierce 660nm Protein Assay Reagent before performing the assay (see above bullet point).

Test Tube Procedure (working range 25-2000µg/mL)

1. Prepare a standard curve within the assay's working range. If using the pre-diluted standards and want a 25µg/mL standard, mix 10µL of the 1000µg/mL standard with 390µL of 0.9% saline and 0.05% of sodium azide.
2. Add 0.1mL of each replicate of standard, unknown sample and the appropriate blank sample into an appropriately labeled test tube.

   Note: A smaller sample volume may be used if the sample to Assay Reagent ratio is maintained at 1:15.
3. Mix Protein Assay Reagent well before use by inverting the bottle several times. Add 1.5mL of the Protein Assay Reagent to each tube and vortex to mix well.
4. Cover and incubate tubes for 5 minutes at room temperature.
5. With the spectrophotometer set to 660nm, zero the instrument on a cuvette filled with only water. Subsequently, measure the absorbance of all the samples.

   Note: If a 660nm filter is not available, measure the assay at any wavelength from 645 to 670nm; however, the assay linear range is 25-2000µg/mL and occurs only when the absorbance is measured at 660nm. Measuring the absorbance at another wavelength will decrease the assay's linear range and might increase the minimum detection level (i.e., decrease sensitivity).
6. Subtract the average 660nm absorbance measurement of the Blank standard replicates from the 660nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 660nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedure (working range 50-2000µg/mL)

1. Prepare a standard curve within the assay's working range. If using the pre-diluted standards and want a 50µg/mL standard, mix 10µL of the 1000µg/mL standard with 190µL of 0.9% saline and 0.05% of sodium azide.
2. Add 10µL of each replicate of standard, unknown sample and the appropriate blank sample into a microplate well (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).
3. Mix Protein Assay Reagent well before use by inverting the bottle several times. Add 150µL of the Protein Assay Reagent to each well.
4. Cover plate and mix on a plate shaker at medium speed for 1 minute. Incubate at room temperature for 5 minutes.
5. Use the blank wells to zero the plate reader. Measure the absorbance of the standards and unknown samples at 660nm.

   Note: If a 660nm filter is not available, measure the assay at any wavelength from 645 to 670nm; however, the assay linear range is 50-2000µg/mL and occurs only when the absorbance is measured at 660nm. Measuring the absorbance at another wavelength will decrease the assay's linear range and might increase the minimum detection level (i.e., decrease sensitivity).
6. Prepare a standard curve by plotting the average Blank-corrected 660nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) curve produces more accurate results than a linear fit.

Troubleshooting

Problem	Possible Cause	Solution
Standards and samples yield lower values than expected	Absorbance measured at incorrect wavelength.	Measure absorbance at 660nm.
Precipitate forms in some tubes	Samples stood for extended time, allowing aggregates to form with the dye.	Mix samples by pipetting up and down immediately before measuring absorbance.
	Sample contained RNA/DNA.	Add a final concentration of 0.8% Triton X-100 to samples.
Blank is >0.25	Sample contained an interfering substance.	Refer to Table 1 for more information.
	Assay reagent was stored at 4°C.	Store the assay reagent at room temperature.
Color of samples appear darker than expected	Protein concentration was too high.	Dilute sample.

Interfering Substances

Certain substances are known to interfere with the Pierce 660nm Protein Assay. Maximum compatible concentrations for many substances are listed in Table 1. Substances were considered compatible in the assay if the error in protein concentration estimation caused by the presence of the substance was ≤ 10%. Blank-corrected 660nm absorbance values for 1mg/mL of BSA plus interfering substance were compared to the net 660nm values of the same standard prepared in water.§

Table 1. Maximum compatible substance concentrations in the Thermo Scientific Pierce 660nm Protein Assay.

Substances	Maximum Compatible Concentration
Detergents	
Tween-20	10%
Triton X-114	0.5%
Triton X-100	1%
Octylthioglucopyranoside	10%
CHAPS	5%
CHAPSO	4%
NP-40	5%
Octyl-β-glucoside	5%
Brij-35	5%
SDS	0.0125%, 5%*
Sodium deoxycholate	0.25%
Zwittergent 3-14	0.05%
CTAB*	2.5%
Cetylpyridinium chloride*	2.5%
DTAB*	2%
Reducing Agents	
DTT	500mM
2-Mercaptoethanol	1M
L-Cysteine	350mM
Ascorbic acid	500mM
TCEP	40mM
Glutathione (reduced)	100mM
Buffers	
PBS	Undiluted
HEPES, pH 7.5	100mM
Tris•HCl, pH 8.0	250mM
Glycine buffer, pH 2.8	100mM
Carbonate-bicarbonate, pH 9.4	diluted 3-fold
Imidazole pH 7.0	200mM
MOPS, pH 7.2	125mM
MES, pH 6.1	125mM
PIPES, pH 6.8	100mM
Sodium acetate, pH 4.8	100mM
Borate buffer, pH 8.5	Undiluted (# 28384)

Substances	Maximum Compatible Concentration
Chelating Agents	
EDTA	20mM
EGTA	20mM
Sodium citrate	12.5mM
Misc Reagents/Solvents	
NaCl	1.25M
GuHCl	2.5M
Urea	8M
Thiourea	2M
Ammonium sulfate	125mM
Glycerol	50%
NaOH	125mM
HCl	125mM
Sucrose	50%
Methanol	50%
Ethanol	50%
DMF	50%
DMSO	50%
Acetone	50%
Acetonitrile	50%
Phenol Red	0.5mg/mL
Calcium chloride in TBS, pH 7.2	40mM
Cobalt chloride in TBS, pH 7.2	20mM
Ferric chloride in TBS, pH 7.2	5mM
Nickel chloride in TBS, pH 7.2	10mM
Zinc chloride in TBS, pH 7.2	10mM
Y-PER™ Reagent	Not compatible
B-PER™ Reagent	diluted 2-fold
M-PER™ Reagent	diluted 2-fold
P-PER™ Reagent	diluted 2-fold
T-PER™ Reagent	diluted 2-fold
MEM-PER™ Reagent	Compatible (1:1:2 of Reagent A:Reagent B:Reagent C)
NE-PER™ Reagent	Compatible (400µL CER I, 22µL CER II and 200µL NER)
2-D Sample Buffer for soluble and insoluble proteins	8M urea, 4% CHAPS, and 7M urea, 2M thiourea, 4% CHAPS
Laemmli SDS sample buffer*	65mM Tris•HCl, 10% glycerol, 2% SDS, 0.0025% bromophenol blue

§For a more extensive list of substances, download Tech Tip # 68: Protein Assay Compatibility Table from our website. This Tech Tip includes compatible substances for all of our protein assays and enables easy comparisons.

*In the presence of 50mM Ionic Detergent Compatible Reagent (IDCR).

Additional Information

A. Response Characteristics for Different Proteins

Each total protein assay method exhibits some degree of varying response toward different proteins. These differences relate to amino acid sequence, isoelectric point, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. The ideal protein to use as a standard in any protein assay is a purified preparation of the protein being assayed. In the absence of a reference protein, use another protein that produces a similar color response to that of the protein being assayed. Most protein methods use BSA or BGG as the standard against which the concentration of protein in the sample is determined (Figure 1). The BSA standard is an appropriate standard if the sample contains primarily albumin. The BGG standard is an appropriate standard if the sample contains gamma globulins.

Typical protein-to-protein variations in color response are listed in Table 2. All proteins were tested at 1mg/mL using the test-tube protocol. The average net color response for BSA was normalized to 1.00 and the average net color response of the other proteins is expressed as a ratio to the response of BSA.

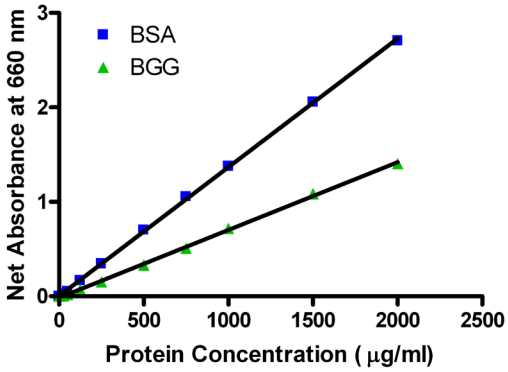

Figure 1. Typical color response curves for BSA and BGG using the test tube procedure.

Table 2. Protein-to-protein variation.

Protein Tested	Ratio
Albumin, bovine serum	1.00
Gamma globulin, bovine	0.51
IgG, human	0.57
IgG, rabbit	0.38
IgG, mouse	0.48
Insulin, bovine pancreas	0.81
Cytochrome c, horse heart	1.22
α-Lactalbumin	0.82
Lysozyme	0.79
Myoglobin, horse heart	1.18
Trypsin inhibitor, soybean	0.38
Ovalbumin	0.54
Transferin, human	0.8
Aldolase	0.83
Average Ratio	**0.7364**
Standard Deviation	**0.2725**
Coefficient of Variation	**37%**

Related Thermo Scientific Products

15041	**Pierce 96-Well Plates – CornerNotch,** 100/pkg
22663	**Ionic Detergent Compatibility Reagent,** 5 × 1g
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set**, 7 × 3.5mL
23209	**Albumin Standard Ampules, 2mg/mL,** 10 × 1mL ampules
23212	**Bovine Gamma Globulin Standard Ampules**, 2mg/mL, 10 × 1mL
23213	**Pre-Diluted Protein Assay Standards: Bovine Gamma Globulin Fraction II**, 7 × 3.5mL

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.