

Pierce™ 660nm Protein Assay

22660 22662

2070.3

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 4°C. 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.

Procedure for the Pierce 660nm Protein Assay

Note: Certain substances interfere with the Pierce 660nm Protein Assay. Please see the Interfering Substances Section for more information.

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).
- For cell lysates prepared in RIPA buffer, add Triton™ X-100 to a final concentration of 0.8% to the sample before performing the assay. For example, to 46µL of control RIPA buffer and diluted lysates (1:10, 1:20 etc), add 4µL of 10% Triton X-100 and mix. Perform the assay as described in the protocol and multiply the protein concentration of the sample by 1.087 (i.e., the dilution factor).

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. 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. 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
A precipitate forms in some tubes	Samples left to stand for extended time, allowing aggregates to form with the dye	Mix samples by pipetting up and down immediately before measuring absorbance
	Sample contains RNA/DNA	Add a final concentration of 0.8% Triton X-100 to samples
Blank is > 0.25	Sample contains an interfering substance	Refer to Table 1 for more information
	Assay reagent is stored at 4°C	Store the assay reagent at room temperature
Color of samples appear darker than expected	Protein concentration is 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 $\leq 10\%$. 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	Substances	Maximum Compatible Concentration
Detergents		Chelating Agents	
Tween-20	10%	EDTA	20mM
Triton X-114	0.5%	EGTA	20mM
Triton X-100	1%	Sodium citrate	12.5mM
Octylthioglucopyranoside	10%	Misc Reagents/Solvents	
CHAPS	5%	NaCl	1.25M
CHAPSO	4%	GuHCl	2.5M
NP-40	5%	Urea	8M
Octyl- β -glucoside	5%	Thiourea	2M
Brij-35	5%	Ammonium sulfate	125mM
SDS	0.0125%, 5%*	Glycerol	50%
Sodium deoxycholate	0.25%	NaOH	125mM
Zwittergent 3-14	0.05%	HCl	125mM
CTAB*	2.5%	Sucrose	50%
Cetylpyridinium chloride*	2.5%	Methanol	50%
DTAB*	2%	Ethanol	50%
Reducing Agents		DMF	50%
DTT	500mM	DMSO	50%
2-Mercaptoethanol	1M	Acetone	50%
L-Cysteine	350mM	Acetonitrile	50%
Ascorbic acid	500mM	Phenol Red	0.5mg/mL
TCEP	40mM	Calcium chloride in TBS, pH 7.2	40mM
Glutathione (reduced)	100mM	Cobalt chloride in TBS, pH 7.2	20mM
Buffers		Ferric chloride in TBS, pH 7.2	5mM
PBS	Undiluted	Nickel chloride in TBS, pH 7.2	10mM
HEPES, pH 7.5	100mM	Zinc chloride in TBS, pH 7.2	10mM
Tris•HCl, pH 8.0	250mM	Y-PER™ Reagent	Not compatible
Glycine buffer, pH 2.8	100mM	B-PER™ Reagent	diluted 2-fold
Carbonate-bicarbonate, pH 9.4	diluted 3-fold	M-PER™ Reagent	diluted 2-fold
Imidazole pH 7.0	200mM	P-PER™ Reagent	diluted 2-fold
MOPS, pH 7.2	125mM	T-PER™ Reagent	diluted 2-fold
MES, pH 6.1	125mM	MEM-PER™ Reagent	Compatible (1:1:2 of Reagent A:Reagent B:Reagent C)
PIPES, pH 6.8	100mM	NE-PER™ Reagent	Compatible (400 μ L CER I, 22 μ L CER II and 200 μ L NER)
Sodium acetate, pH 4.8	100mM	RIPA buffer	50mM Tris•HCl, 150mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1mM EDTA
Borate buffer, pH 8.5	Undiluted (# 28384)	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.

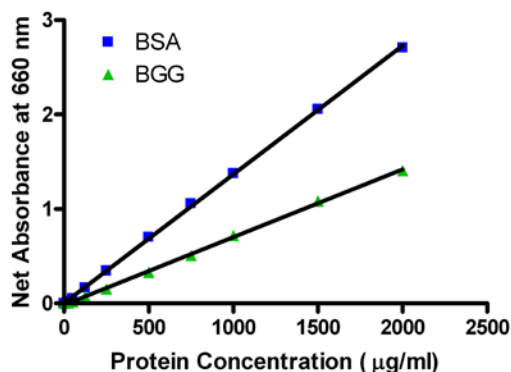


Figure 1. Typical color response curves for BSA and BGG using the test tube procedure.

Table 2. Protein-to-protein variation.

<u>Protein Tested</u>	<u>Ratio</u>
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

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