

Pierce[®] 660 nm Protein Assay

22660 22662

2070.1

Number	Description
22660	Pierce 660 nm Protein Assay Reagent , 750 ml, contains sufficient reagents for 500 test tube or 5,000 microplate assays
22662	Pierce 660 nm Protein Assay Kit , sufficient reagents for 300 test tube or 3,000 microplate assays Kit Contents: Pierce 660 nm Protein Assay Reagent , 450 ml Pre-diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set , 7 × 3.5 ml, contains standardized BSA solutions at a specific concentration from 125 to 2,000 µg/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 660 nm 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 660 nm Protein Assay

Note: Certain substances interfere with the Pierce 660 nm 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 20 ml of the Pierce 660 nm 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 20 ml of the Pierce 660 nm 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 46 µl of control RIPA buffer and diluted lysates (1:10, 1:20 etc), add 4 µl 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 25-2,000 µg/ml)

1. Prepare the 25 µg/ml standard by mixing 10 µl of 1,000 µg/ml BSA standard with 390 µl of 0.9% saline and 0.05% of sodium azide.
2. Pipette 0.1 ml 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 1.5 ml 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 660 nm, zero the instrument on a cuvette filled with only water. Subsequently, measure the absorbance of all the samples.

Note: If a 660 nm filter is not available, measure the assay at any wavelength from 645 to 670 nm; however, the assay linear range is 25-2,000 µg/ml and occurs only when the absorbance is measured at 660 nm. 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 660 nm absorbance measurement of the Blank standard replicates from the 660 nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 660 nm 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 50-2,000 µg/ml)

1. Prepare the 50 µg/ml standard by mixing 10 µl of 1,000 µg/ml BSA standard with 190 µl of 0.9% saline and 0.05% of sodium azide.
2. Pipette 10 µl of each replicate of standard, unknown sample and the appropriate blank sample into a microplate well.
3. Add 150 µl 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 660 nm.

Note: If a 660 nm filter is not available, measure the assay at any wavelength from 645 to 670 nm; however, the assay linear range is 50-2,000 µg/ml and occurs only when the absorbance is measured at 660 nm. 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 660 nm 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 660 nm
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 660 nm 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 660 nm absorbance values for 1 mg/ml of BSA plus interfering substance were compared to the net 660 nm values of the same standard prepared in water.

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

Substances	Maximum Compatible Concentration	Substances	Maximum Compatible Concentration
Detergents		Chelating Agents	
Tween-20	10%	EDTA	20 mM
Triton X-114	0.5%	EGTA	20 mM
Triton X-100	1%	Sodium citrate	12.5 mM
Octylthioglucopyranoside	10%	Misc Reagents/Solvents	
CHAPS	5%	NaCl	1.25 M
CHAPSO	4%	GuHCl	2.5 M
NP-40	5%	Urea	8 M
Octyl- β -glucoside	5%	Thiourea	2 M
Brij-35	5%	Ammonium sulfate	125 mM
SDS	0.0125%, 5%*	Glycerol	50%
Sodium deoxycholate	0.25%	NaOH	125 mM
Zwittergent 3-14	0.05%	HCl	125 mM
CTAB*	2.5%	Sucrose	50%
Cetylpyridinium chloride*	2.5%	Methanol	50%
DTAB*	2%	Ethanol	50%
Reducing Agents		DMF	50%
DTT	500 mM	DMSO	50%
2-Mercaptoethanol	1 M	Acetone	50%
L-Cysteine	350 mM	Acetonitrile	50%
Ascorbic acid	500 mM	Phenol Red	0.5 mg/ml
TCEP	40 mM	Calcium chloride in TBS, pH 7.2	40 mM
Glutathione (reduced)	100 mM	Cobalt chloride in TBS, pH 7.2	20 mM
Buffers		Ferric chloride in TBS, pH 7.2	5 mM
PBS	Undiluted	Nickel chloride in TBS, pH 7.2	10 mM
HEPES, pH 7.5	100 mM	Zinc chloride in TBS, pH 7.2	10 mM
Tris•HCl, pH 8.0	250 mM	Y-PER [®] Reagent	Not compatible
Glycine buffer, pH 2.8	100 mM	B-PER [®] Reagent	diluted 2-fold
Carbonate-bicarbonate, pH 9.4	diluted 3-fold	M-PER [®] Reagent	diluted 2-fold
Imidazole pH 7.0	200 mM	P-PER [®] Reagent	diluted 2-fold
MOPS, pH 7.2	125 mM	T-PER [®] Reagent	diluted 2-fold
MES, pH 6.1	125 mM	MEM-PER [®] Reagent	Compatible (150 μ l Reagent A, 150 μ l Reagent B and 300 μ l Reagent C)
PIPES, pH 6.8	100 mM	NE-PER [®] Reagent	Compatible (400 μ l CER I, 22 μ l CER II and 200 μ l CER)
Sodium acetate, pH 4.8	100 mM	RIPA buffer	50 mM Tris•HCl, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA
Borate buffer, pH 8.5	Undiluted (# 28384)	2-D Sample Buffer for soluble and insoluble proteins	8 M urea, 4% CHAPS, and 7 M urea, 2 M thiourea, 4% CHAPS
		Laemmli SDS sample buffer*	65 mM Tris•HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol blue

*In the presence of 50 mM 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 1 mg/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.

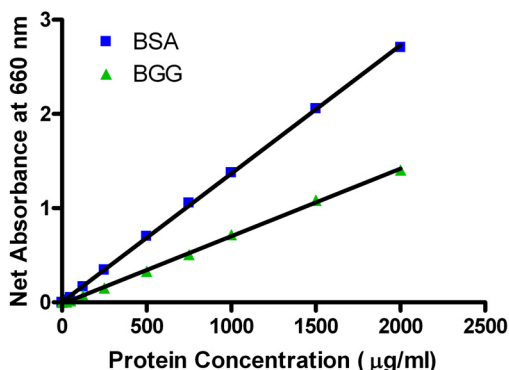


Figure 1. Typical color response curves for BSA and BGG using the test tube procedure.

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 Products

- 22663 **Ionic Detergent Compatibility Reagent, 5 × 1 g**
- 23208 **Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set, 7 × 3.5 ml**
- 23209 **Albumin Standard Ampules, 2 mg/ml, 10 × 1 ml ampules**
- 23212 **Bovine Gamma Globulin Standard Ampules, 2 mg/ml, 10 × 1 ml**
- 23213 **Pre-Diluted Protein Assay Standards: Bovine Gamma Globulin Fraction II (BGG) Set, 7 × 3.5 ml**

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