Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible

Catalog Numbers A55866

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible is a quick and ready-to-use modification of the well-known Bradford Coomassie-binding, colorimetric method for total protein quantitation. When Coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs (465 nm to 595 nm) with a concomitant color change from brown to blue. This kit provides a set of pre-diluted bovine serum albumin (BSA) protein standards in a convenient eight-channel tubestrip that is compatible with a multichannel pipette allowing all protein standards and a blank solution to be transferred to a microplate simultaneously.

Performing the assay is simple: combine a small amount of protein sample with the assay reagent, mix well, incubate briefly, then measure the absorbance at 595 nm. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions that are assayed alongside the unknown samples. Because the color response with Coomassie is non-linear with increasing protein concentrations, a standard curve must be completed with each assay.

Contents and storage

Table 1 Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, Cat. No. A55866

Contents	Storage
950 mL kit sufficient for 3,160 microplate assays or 630 test-tube assays **Contents:** Pierce™ Bradford Plus Protein Assay Reagent, 950 mL, contains Coomassie G-250 dye, methanol, phosphoric acid, and solubilizing agents in water Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL, 2 x 1 mL, 8-channel tubestrip containing bovine serum albumin (BSA) at concentrations referenced in Figure 2 in 0.9% saline and 0.05% sodium azide.	4°C

Note: Discard any reagent that shows discoloration or evidence of microbial contamination.

Workflow

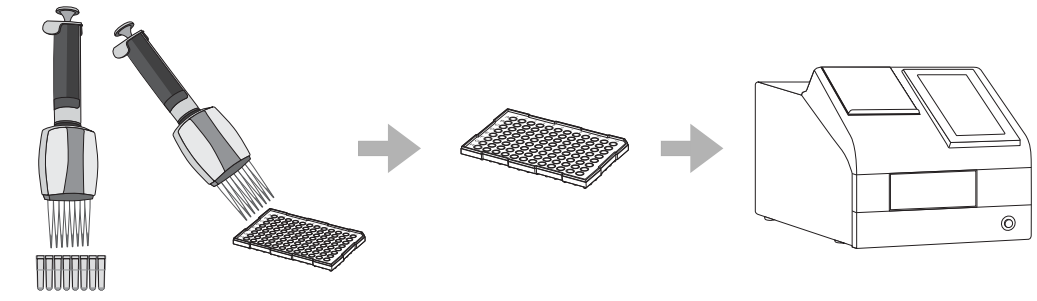

Fig. 1 Protocol summary

Prepare BSA standards

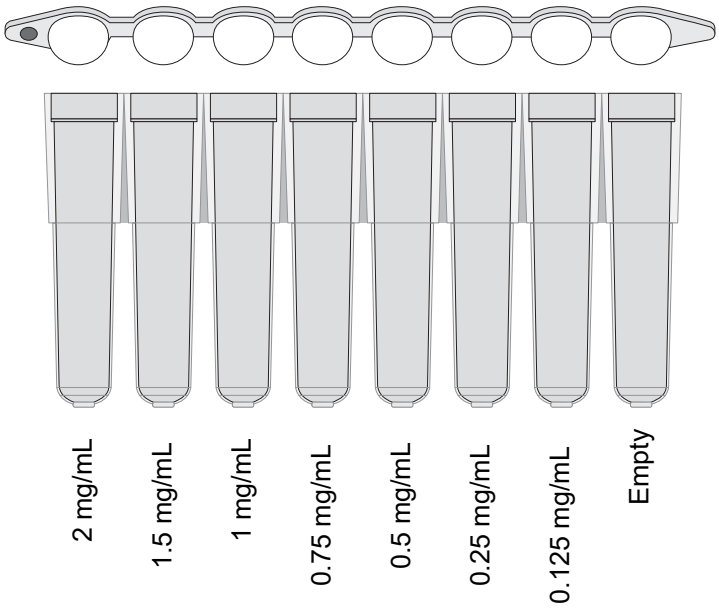

Fig. 2 Pierce™ Dilution-Free™ BSA protein standards tubestrip format indicating the BSA protein concentration of each tube

Each tube of the tubestrip contains 1 mL of corresponding BSA concentration in a format compatible with a multichannel pipette. One tube is supplied empty allowing for the addition of a blank solution.

1. When ready to use, carefully remove foil from the tubestrip.
2. After foil is removed, the provided strip cap will be used to reseal the tubes. To keep the strip cap oriented there is a small hole at one end of the cap strip which should be aligned with the 2 mg/mL standard. The tubestrip has a single label on the tube containing the 2 mg/mL solution.
3. Push the cap strip firmly onto the tubestrip. Ensure that each of the 8 caps fit securely in each of the 8 tubes.
4. Store at 4°C.

Prepare Bradford Plus Reagent

Mixing, then equilibrating the Bradford Plus Reagent

1. Mix the Bradford Plus Reagent solution immediately before use by gently inverting the bottle several times.
   Do not shake the bottle to mix the solution.
2. Remove the amount of reagent needed and equilibrate to room temperature (RT) before use.
   Note: Pierce™ Bradford Plus Protein Assay Reagent contains additives which will retard formation of dye-dye and dye-protein aggregates that tend to form in all coomassie-based protein assay reagents. If left undisturbed, the aggregates will become large enough to be visible. When left overnight in a clear glass tube, the reagent forms dye-dye aggregates that are visible as a dark precipitate in the bottom of the tube with a nearly colorless liquid above. While dye-dye aggregates can form over several hours in stored reagent, dye-protein-dye aggregates form more quickly. Gentle mixing will completely disperse the dye-dye aggregates. It is recommended that the Bradford Plus Reagent be mixed before pipetting and that each plate be mixed immediately before measuring absorbances.

Microplate procedure

Standard microplate procedure

Working range = 125-1,500 µg/mL

1. Pipette 5 µL of each standard or unknown sample into the appropriate microplate wells (for example, Thermo Scientific™ Pierce™ 96-well plates, Cat. No. 15041).
2. Add 250 µL of the Bradford Plus Reagent to each well, then mix with plate shaker for 30 seconds.
3. Remove plate from shaker. Incubate plate for 10 minutes at room temperature.
4. Measure the absorbance with a plate reader at, or near, 595 nm.
5. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595 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) or best-fit curve will provide more accurate results than a purely linear fit.

Micro microplate procedure

Working range = 1-25 µg/mL

1. Pipette 150 µL of each standard or unknown sample into the appropriate microplate wells.
2. Add 150 µL of the Bradford Plus Reagent to each well, then mix with plate shaker for 30 seconds.
3. Remove plate from shaker. Incubate plate for 10 minutes at room temperature.
4. Measure the absorbance with a plate reader at, or near, 595 nm.
5. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595 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) or best-fit curve will provide more accurate results than a purely linear fit.

Related products

Product	Cat. no.
Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL	A55863
Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit	A55860
Pierce™ 96-Well Polystyrene Plates, Corner Notch, 100/pkg	15041
ELISA Reagent Reservoirs , 200/pkg	15075
Sealing Tape for 96-Well Plates, 100/pkg	15036
Pierce™Bovine Serum Albumin Standard Ampules, 2 mg/mL, 10 x 1 mL	23209
Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set, 7 x 3.5 mL	23208
Pierce™ Bovine Gamma Globulin Standard Ampules, 2 mg/mL, 10 x 1 mL	23212
Pierce™ Bovine Gamma Globulin Standard Pre-Diluted Set, 7 x 3.5 mL	23213
Pierce™ Detergent Compatible Bradford Assay Kit	23246
Micro BCA™ Protein Assay Kit, working range 0.5-20 µg/mL	23235
Pierce™ BCA Protein Assay Kit, working range of 20-2000 µg/mL	23227
Compat-Able™ Protein Assay Preparation Reagent Set	23215

Troubleshooting

Observation	Possible cause	Recommended action
Absorbance of blank is acceptable, but standards and samples yield lower values than expected	Reagent was stored improperly.	Refrigerate stored reagent.
	Reagent was cold.	Allow reagent to warm to room temperature.
	Incorrect wavelength was used to measure absorbance.	Measure absorbance near 595 nm.
Absorbances of blank and standards are acceptable, but samples show less color than expected	Sample protein (peptide) had a molecular weight < 3,000 daltons.	Use the Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit or the Pierce™ BCA Assay Kit.
A precipitate forms in all tubes	Sample contained a surfactant (detergent).	Dialyze or dilute the sample.
	Samples were not mixed well or were left to stand for an extended time, allowing aggregates to form with the dye.	Remove interfering substances. See “Additional information” on page 3.
All tubes (including blank) are dark blue in color	Strong alkaline buffer raised pH of formulation, or the sample volume was too large, raising the reagent pH.	Dialyze or dilute sample.
		Remove interfering substances. See “Additional information” on page 3.
Cannot measure color at a specific wavelength	Spectrophotometer or plate reader did not have 595 nm filter.	Color may be measured at any wavelength between 575—615 nm, but the standard curve slope and overall assay sensitivity will be reduced.

Additional information

Interfering substances

Certain substances are known to interfere with Coomassie-based protein assays including most ionic and nonionic detergents which reduce color development and can cause precipitation of the assay reagent. Other substances interfere to a lesser extent. Maximum compatible concentrations for many substances are listed in Table 2.

The effects of interfering substances may be overcome by several methods:

- Remove the interfering substance by dialysis or desalting.
- Dilute the sample until the substance no longer interferes.

- Precipitate proteins with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interfered should be discarded and the protein pellet solubilized in a small amount of ultrapure water or in the Bradford Plus Reagent.
- Remove the interfering substance using Compat-Able™ Protein Assay Preparation Reagent Set, Cat. No. 23215.

Response characteristics for different proteins

Each of the commonly used total protein assay methods 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 color response of the protein.

Most protein assay methods utilize BSA or immunoglobulin (IgG) as the standard to determine the concentration of protein in the sample. Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL (A55863) provide a convenient standard for protein estimations. However, individual proteins, including BSA and IgG, differ slightly in their color responses in the Bradford Assay (see Figure 3). For greatest accuracy, the standard curve should be prepared from a pure sample of the target protein to be measured.

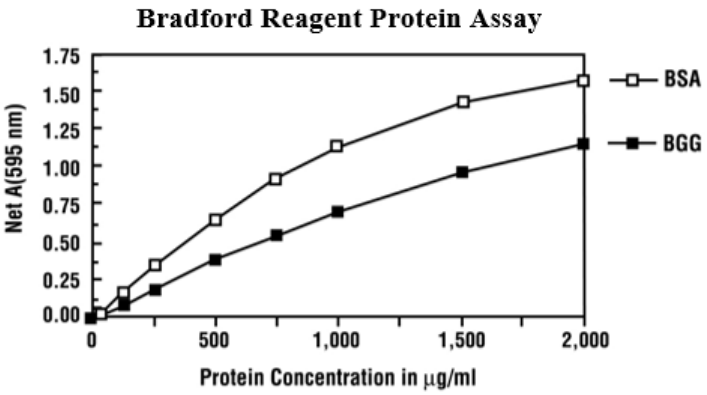

Fig. 3 Typical color response curves for BSA and BGG using the standard protocol of the Bradford Plus Assay.

Measuring absorbances at wavelengths other than 595 nm

If a spectrophotometer or plate reader is not available with a 595 nm filter, the blue color may be measured at any wavelength between 570–610 nm. However, the maximum sensitivity of the assay occurs when the absorbance of the dye-protein complex is measured at 595 nm. Measuring the absorbance at any wavelength other than 595 nm will result in a lower slope for the standard curve and may increase the minimum detection level for the protocol.

Effect of temperature on 595 nm absorbance

Absorbance measurements at 595 nm obtained with the Bradford Plus Reagent are somewhat dependent on the temperature of the reagent. As the reagent temperature increases to room temperature, the 595 nm measurements will increase. The Bradford Reagent should remain at a constant temperature (i.e., room temperature) during the assay.

Assay compatibility for various substances

Note: For a more extensive list of substances, download *Tech Tip: Protein Assay Compatibility Table* from our website. This tech tip includes compatible substances for many of our protein assays and enables easy comparisons.

Table 2 Compatible substance concentrations in the Bradford Plus Assay

Salts/Buffers	Compatible Concentration
ACES, pH 7.8	100 mM
Ammonium sulfate	1 M
Asparagine	10 mM
Bicine, pH 8.4	100 mM
Bis-Tris, pH 6.5	100 mM
Borate (50 mM), pH 8.5 (Cat. No. 28384)	undiluted
B-PER™ Reagent (Cat. No. 78248)	1:2 dilution[1]
Calcium chloride in TBS, pH 7.2	10 mM
Na-Carbonate/Na-Bicarbonate (0.2 m), pH 9.4 (Cat. No. 28382)	undiluted
Cesium bicarbonate	100 mM
CHES, pH 9.0	100 mM
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (Cat. No. 28386)	undiluted
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (Cat. No. 28388)	undiluted
Cobalt chloride in TBS, pH 7.2	10 mM
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
Glycine	100 mM
Guanidine•HCl	3.5 M
HEPES, pH 7.5	100 mM
Imidazole,pH 7.0	200 mM
MES, pH 6.1	100 mM
MES (0.1M), NaCl (0.9%), pH 4.7 (Cat. No. 28390)	undiluted
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4 (Cat. No. 28374)	undiluted
Nickel chloride in TBS, pH 7.2	10 mM
PBS: Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (Cat. No. 28372)	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer: 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1:10 dilution[1]
Sodium acetate, pH 4.8	180 mM
Sodium azide	0.5%
Sodium bicarbonate	100 mM
Sodium chloride	5 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium phosphate	100 mM
Tricine,pH 8.0	100 mM
Triethanolamine,pH 7.8	100 mM
Tris	2 M
TBS:Tris (25 mM), NaCl (0.15 M), pH 7.6 (Cat. No. 28376)	undiluted
Tris (25 mM), Glycine (192 mM), pH 8.0 (Cat. No. 28380)	undiluted
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3 28378	1:2 dilution[1]
Zinc chloride in TBS, pH 7.2	10 mM

[1] Diluted with ultrapure water

Detergents	Compatible Concentration
Brij™-35	0.125%
Brij™-56, Brij™-58	0.031%
CHAPS, CHAPSO	5.0%
Deoxycholic acid	0.05%
Lubrol™ PX	0.125%
Octyl β-glucoside	0.5%
Octyl β-thioglucopyranoside	3%
Nonidet P-40 (NP-40)	0.5%
SDS	0.125%
Span™-20	0.5%
Triton-X™-100, Triton-X™-114	0.125%
Triton-X™-305, Triton-X™-405	0.5%
Tween™-20, Tween™-80	0.062%
Tween™-60	0.1%
Zwittergent™ 3-14	0.025%

Chelating Agents	Compatible Concentration
EDTA	100 mM
EGTA	2 mM
Sodium citrate	200 mM

Reducing and Thiol-containing Agents	Compatible Concentration
N-acetylglucosamine in PBS, pH 7.2	100 mM
Ascorbic acid	50 mM
Cysteine	10 mM
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	5 mM
Glucose	1 M
Melibiose	100 mM
2-Mercaptoethanol	1 M
Potassium thiocyanate	3 M
Thimerosal	0.01%

Misc. Reagents and Solvents	Compatible Concentration
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF, DMSO	10%
Ethanol	10%
Glycerol (fresh)	10%
Hydrochloric acid	100 mM
Leupeptin	10 mg/L
Methanol	10%
Phenol Red	0.5 mg/mL
PMSF	1 mM
Sodium Hydroxide	100 mM
Sodium vanadate (sodium salt), in PBS, pH 7.2	1 mM
Sucrose	10%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Urea	3 M

References

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

Revision history: Pub. No. MAN0029425 A.0

Revision	Date	Description
A.0	17 July 2023	New document for Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible .

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