Pierce™ Bradford Protein Assay Kit

Catalog Number 23200

Pub. No. MAN0011181 Rev. C.0

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 Protein Assay Kit 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 from 465 nm to 595 nm with a concomitant color change from brown to blue.

Performing the assay in either a test tube or microplate format 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, which are assayed alongside the unknown samples. Because the color response with Coomassie is non-linear with increasing protein concentration, a standard curve must be completed with each assay.

Contents and Storage

The Pierce™ Bradford Protein Assay Kit (Cat. No. 23200) contains sufficient reagents for 630 test tube assays or 3,800 microplate assays

Contents	Storage[1]
Pierce™ Bradford Protein Assay Reagent, 950 mL containing Coomassie G-250 dye, methanol, phosphoric acid, and solubilizing agents in water.	4°C
Albumin Standard Ampules, 10 x 1 mL ampules containing bovine serum albumin (BSA) at a concentration of 2 mg/mL in a solution 0.9% saline and 0.05% sodium azide. [2].	Store unopened ampules at room temperature (RT).

[1] Product shipped at ambient temperature.
[2] Available separately as Cat. No. 23209

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

Workflow

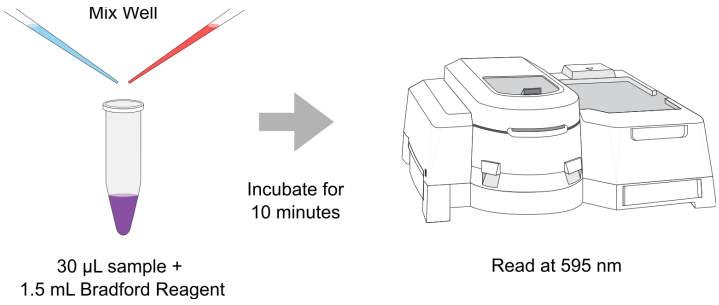

Figure 1 Procedure summary

Preparation of standards and assay reagent

Mix, then equilibrate the Bradford reagent

1. Mix the Bradford reagent solution immediately before use by gently inverting the bottle several times. Do not shake the bottle to mix the solution.

   Note: Dye-dye and dye-protein aggregates tend to form in all coomassie-based 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 nearly colorless liquid above. While dye-dye aggregates can form over several hours in stored reagent, dye-protein aggregates form more quickly. Gentle mixing completely disperses the dye-dye aggregates. It is good practice to mix the Bradford reagent before pipetting and to mix each tube or plate immediately before measuring absorbances.

2. Remove the amount of reagent needed and equilibrate it to room temperature before use.

Prepare BSA standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard ampule into several clean vials, preferably in the same diluent as the sample(s). Each 1 mL ampule of BSA standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard.

Table 1 Preparation of BSA standards

Dilution scheme for standard test tube and microplate protocols (working range = 100–1500 µg/mL)			
Vial	**Volume of diluent**	**Volume and source of BSA**	**Final BSA concentration**
A	0	300 µL of Stock	2,000 µg/mL
B	125 µL	375 µL of Stock	1,500 µg/mL
C	325 µL	325 µL of Stock	1,000 µg/mL
D	175 µL	175 µL of vial B dilution	750 µg/mL
E	325 µL	325 µL of vial C dilution	500 µg/mL
F	325 µL	325 µL of vial E dilution	250 µg/mL
G	325 µL	325 µL of vial F dilution	125 µg/mL
H	400 µL	100 µL of vial G dilution	25 µg/mL
I	400 µL	0	0 µg/mL = Blank
Dilution scheme for micro test tube or microplate protocols (working range = 1–25 µg/mL)			
Vial	**Volume of diluent**	**Volume and source of BSA**	**Final BSA concentration**
A	2,370 µL	30 µL of Stock	25 µg/mL
B	4,950 µL	50 µL of Stock	20 µg/mL
C	3,970 µL	30 µL of Stock	15 µg/mL
D	2,500 µL	2,500 µL of vial B dilution	10 µg/mL
E	2,000 µL	2,000 µL of vial D dilution	5 µg/mL
F	1,500 µL	1,500 µL of vial E dilution	2.5 µg/mL
G	5,000 µL	0	0 µg/mL = Blank

Test tube procedures

Standard test tube protocol

Working range = 100-1500 µg/mL

1. Pipette 0.03 mL (30 µL) of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5 mL of the Bradford reagent to each tube, then mix well.
3. Incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water.
5. Measure the absorbance of all the samples.
6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
7. 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.

Micro test tube protocol

Working range = 1-25 µg/mL

1. Pipette 1.0 mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.0 mL of the Bradford reagent to each tube, then mix well.
3. Incubate samples for 10 minutes at RT.
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water.
5. Measure the absorbance of all the samples.
6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
7. 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.

Microplate procedures

When compared to the standard test tube protocol, 595 nm measurements obtained with the microplate protocols are lower because the light path used is shorter. This may increase the minimum detection level of the assay. If higher 595 nm measurements are required, use 7-10 µL of standard or sample and 250 µL of Bradford reagent per well.

Standard microplate protocol

Working range = 100-1500 µg/mL

1. Pipette 5 µL of each standard or unknown sample into the appropriate microplate wells (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Cat. No. 15041).
2. Add 250 µL of the Bradford reagent to each well, then mix with plate shaker for 30 seconds.
3. Remove the plate from the shaker.
4. Incubate the plate for 10 minutes at RT.
5. Measure the absorbance at or near 595 nm with a plate reader.
6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.

7. 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. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Micro microplate protocol

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 reagent to each well, then mix with plate shaker for 30 seconds.
3. Remove plate from shaker.
4. Incubate plate for 10 minutes at room temperature (RT).
5. Measure the absorbance at or near 595 nm on a plate reader.
6. Subtract the average 595 nm measurement for the blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average blank corrected 595 nm measurement for each BSA standard vs. its concentration in µg/mL. Using the standard curve, determine the protein concentration estimate for 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. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Related Thermo Scientific™ products

Contents	Cat. No.
Pierce™ 96-Well Polystyrene Plates – Corner Notch, 100/pkg	15041
Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL	A55863
Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set	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™ BCA Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, working range of 20-2000 µg/mL	A55864
Micro BCA™ Protein Assay Kit, working range 0.5-20 µg/mL	23235
Compat-Able™ Protein Assay Preparation Reagent Set, sufficient reagents to pre-treat 500 samples to remove interfering substances prior to total protein quantitation	23215
Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit	A55860
Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible	A55866

Troubleshooting

Observation	Possible cause	Recommended action
Standards and samples yield lower values than expected, while absorbance of blank is acceptable	Reagent was stored improperly.	Refrigerate stored reagent.
	The reagent was too cold.	Allow reagent to warm to RT.
	The absorbance was measured at an incorrect wavelength.	Measure absorbance near 595 nm.

Observation	Possible cause	Recommended action
Samples yield lower values than expected, while absorbances of blank and standards are acceptable	The molecular weight of the sample protein (peptide) was too low (e.g., less than 3000 Da).	Use the Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit or the Pierce™ BCA Protein Assay Kit.
A precipitate forms in all tubes	Sample contained a surfactant (detergent).	Dialyze or dilute the sample.
		Remove interfering substances form the samples using Compat-Able™ Protein Assay Preparation Reagent Set, Cat. No. 23215 (see "Interfering substances" on page 5).
All tubes (including blanks) are dark blue	The sample volume was too large or a strong alkaline buffer was present, raising the pH of the formulation.	Dialyze or dilute the sample.
		Remove interfering substances form the samples using Compat-Able™ Protein Assay Preparation Reagent Set, Cat. No. 23215 (see "Interfering substances" on page 5).
Need to read absorbances at a different wavelength	Spectrophotometer or plate reader did not have a 595 nm filter.	The color may be read at any wavelength between 575 nm and 615 nm. However, the slope of the standard curve 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. These have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the standard test tube protocol are listed in "Assay compatibility for various substances" on page 6. Substances were compatible in the standard test tube protocol if the error in protein concentration estimation (of BSA at 1000 µg/mL) caused by the presence of the substance in the sample was less than or equal to 10%. The blank-corrected 595 nm absorbance measurements (for the 1000 µg/mL BSA standard + substance) were compared to the net 595 nm absorbances of the 1000 µg/mL BSA standard prepared in 0.9% saline.

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 Reagent.

  Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).
- Remove the interfering substance using Compat-Able™ Protein Assay Preparation Reagent Set (Cat. No. 23215).

Effect of temperature on 595 nm absorbance

Absorbance measurements at 595 nm obtained with the Bradford reagent are dependent on the temperature of the reagent to some extent. As the reagent temperature increases to room temperature, the 595 nm measurements will increase. Therefore, it is important that the Bradford reagent remain at a constant temperature (i.e., RT) during the assay.

Measuring absorbances at wavelengths other than 595 nm

If a photometer or plate reader is not available with a 595 nm filter, the blue color may be measured at any wavelength between 570 nm and 610 nm. 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.

Cleaning and re-using glassware

Care must be exercised when cleaning glassware that will be used again for protein assays. Thorough cleaning often requires the use of a detergent, such as PCC-54™ Detergent Concentrate (Cat. No. 72288), which must be completely removed in the final rinse. The Coomassie dye will stain glass or quartz cuvettes. Disposable polystyrene cuvettes are a convenient alternative.

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 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

Salts/Buffers	Compatible Concentration
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[1]	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%

[1] Detergents were tested using high-purity Thomas Scientific™ Surfact-Amps™ products which have a low peroxide content.

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

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 against which the concentration of protein in the sample is determined. Albumin Standard Ampules (BSA) (Cat. No. 23209) provide a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Bradford Assay (see Figure 2). For greatest accuracy, the standard curve should be prepared from a pure sample of the target protein to be measured.

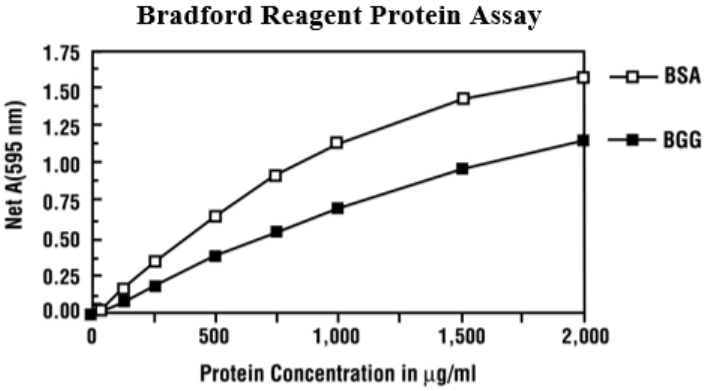

Figure 2 Typical color response curves for BSA and bovine gamma globulin (BGG) using the standard test tube protocol of the Bradford assay.

Table 3 Protein-to-protein variation: Absorbance ratios (595 nm) for proteins relative to BSA using the Standard Test Tube Protocol in the Bradford Assay.

Protein tested	Ratio[1]
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.76
α-Chymotrypsinogen, bovine	0.48
Cytochrome C, horse heart	1.07
Gamma globulin, bovine	0.56
IgG, bovine	0.58
IgG, human	0.63
IgG, mouse	0.59
IgG, rabbit	0.37
IgG, sheep	0.53
Insulin, bovine pancreas	0.60
Myoglobin, horse heart	1.19
Ovalbumin	0.32
Transferrin, human	0.84
Average Ratio	**0.68**
Standard Deviation	**0.26**
Coefficient of Variation	**38.2%**

[1] Ratio = (Avg "test" net Abs.)/(avg. BSA net Abs.)

Table 3 shows typical protein-to-protein variation in color response. All proteins were tested at a concentration of 1000 µg/mL using the Standard 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. The protein-to-protein variation observed with the Bradford reagent is significantly less than that seen with other Bradford-type Coomassie dye formulations.

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. MAN0011181 C.0

Revision	Date	Description
C.0	17 July 2023	New document for Bradford Protein Assay Kit.

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