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

Catalog Numbers A55864 and A55865

Pub. No. MAN0029423 Rev. B.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™ BCA Protein Assay Kit with Dilution-Free™ BSA Protein Standards is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing BCA.

The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20–2,000 µg/mL).

The BCA method is not a true end-point method. The final color continues to develop. However, the rate of continued color development is sufficiently slow after incubation to allow large numbers of samples to be assayed together. Protein concentrations are generally determined and reported with reference to standards of a common protein, such as bovine serum albumin (BSA).

This kit comes with pre-diluted BSA protein standards in a convenient eight channel tube set that is compatible with a multi-channel pipette and allows all protein standards and blank solution to be transferred to a microplate simultaneously. If precise quantitation of an unknown protein is required, it is advisable to select a protein standard that is similar in quality to the unknown, such as a bovine gamma globulin (BGG) standard (see “Related products” on page 4) which may be used when assaying immunoglobulin samples.

Note: For peptide sample concentration measurements, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay Kit (Cat. No. 23290) or the Pierce™ Quantitative Colorimetric Peptide Assay Kit (Cat. No. 23275). See “Related products” on page 4.

Workflow

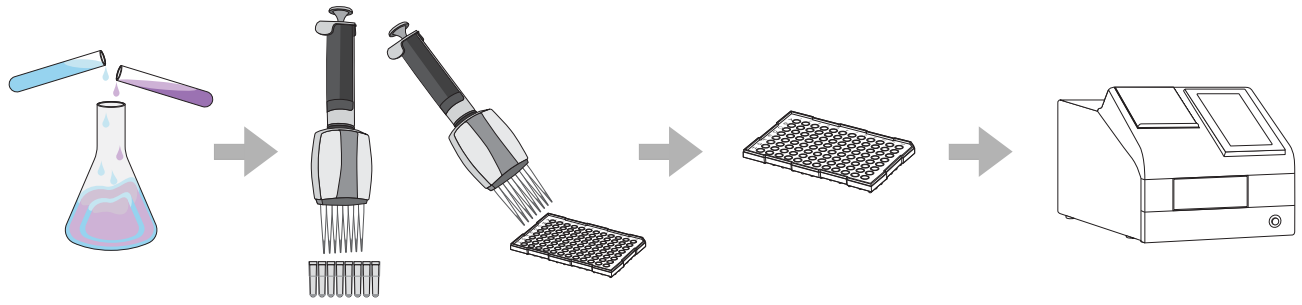

Figure 1 Procedure summary

Contents

Table 1 Pierce™ BCA Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible

Cat. No.	Contents	Storage
A55864	1 L kit sufficient for 5,000 microplate assays or 500 cuvette assays, contains: **BCA Assay Reagent A**, 2 x 500 mL **BCA Assay Reagent B**, 1 x 25 mL **Dilution-Free BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL**, 4 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.	Room temperature
A55865	500 mL kit sufficient for 2,500 microplate assays or 250 cuvette assays, contains: **BCA Assay Reagent A**, 1 x 500 mL **BCA Assay Reagent B**, 1 x 25 mL **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	

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

Prepare BSA standards (required for both assay procedures)

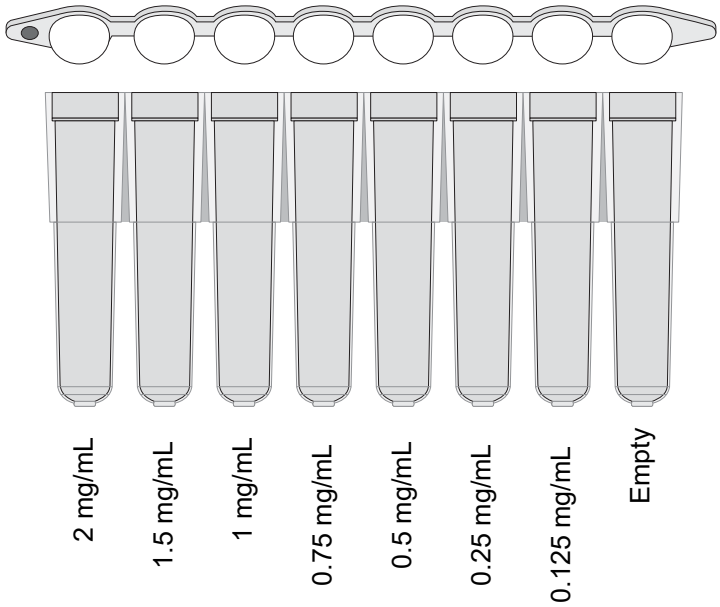

Figure 2 Pierce™ Dilution-Free™ BSA protein standards tubestrip format indicating the BSA protein concentration of each tube.

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 BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   (# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume WR required

   Example (for the microplate procedure with 10 unknowns and 2 replicates of each sample): (8 standards + 10 unknowns) x (2 replicates) x (0.2 mL) = 7.2 mL

   Prepare 10 mL of WR to ensure enough WR is available for the assay.

2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

   Note: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing. The resulting WR will be clear and green.

Perform microplate procedure

1. Pipette 25 µL of each standard or unknown sample replicate into a microplate well (working range = 20-2,000 µg/mL) (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).

   Note: If sample size is limited, 10 µL of each unknown sample and standard can be used (sample to WR ratio = 1:20). However in this case, the working range of the assay is limited to 125-2,000 µg/mL.

2. Add 200 µL of the WR to each well, then mix plate thoroughly on a plate shaker for 30 seconds.

3. Cover plate and incubate at 37°C for 30 minutes. Cool plate to room temperature.

4. Measure the absorbance at or near 562 nm on a plate reader.

   Note: Wavelengths of 540 - 590 nm have been used successfully with this method.

5. Subtract the average 562 nm absorbance measurement of the blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.

6. Prepare a standard curve by plotting the average blank-corrected 562 nm absorbance measurement for each BSA standard versus its concentration in µg/mL.

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

Perform cuvette procedure

1. Pipette 0.1 mL of each standard and unknown sample replicate into an appropriately labeled cuvette.

2. Add 2.0 mL of the WR to each cuvette, then mix well.

3. Cover and incubate cuvettes at selected temperature and time based on the protocols below.
   - Standard protocol: 37°C for 30 minutes (working range = 20–2,000 µg/mL). Use a hot water bath to heat the cuvettes. Do not use a forced-air incubator as this can introduce significant error in color development because of uneven heat transfer.
   - Room temperature (RT) protocol: RT for 2 hours (working range = 20–2,000 µg/mL). Use a hot water bath to heat the cuvettes. Do not use a forced-air incubator as this can introduce significant error in color development because of uneven heat transfer.
   - Enhanced protocol: 60°C for 30 minutes (working range = 5–250 µg/mL).

   Note: Increasing the incubation time or temperature increases the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.

4. Cool all cuvettes to RT.

5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Measure the absorbance of all the samples within 10 minutes.

   Note: Because the BCA assay does not reach a true end point, color development will continue even after cooling to RT. However, the rate of color development is low at RT. No significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.

6. Subtract the average 562 nm absorbance measurement of the blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.

7. Prepare a standard curve by plotting the average blank-corrected 562 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.

Related products

Product	Cat. no.
Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set, 7 x 3.5 mL	23208
Pierce™ Bovine Gamma Globulin Standard Pre-Diluted Set, 7 x 3.5 mL	23213
Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL	A55863
Pierce™ Bovine Serum Albumin Standard Ampules, 2 mg/mL, 10 x 1 mL	23209
Pierce™ Bovine Gamma Globulin Standard Ampules, 2 mg/mL, 10 x 1 mL	23212
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™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit	A55860
Pierce™ BCA Protein Assay Kit - Reducing Agent Compatible	23250
Micro BCA™ Protein Assay Kit	23235
Compat-Able™ Protein Assay Preparation Reagent Set	23215
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275

Additional information

Alternative total protein assay reagents

If interference by a reducing substance or metal-chelating substance in the sample cannot be overcome, try Pierce™ Bradford Plus Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible (Cat. No. A55866) which is less sensitive to such substances. See Table 3.

Cleaning and reusing glassware

Exercise care when reusing glassware. All glassware must be cleaned and given a thorough final rinse with ultrapure water.

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, 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 use BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined (see Figure 3). To obtain greater accuracy, prepare the standard curve from a pure sample of the target protein.

Typical protein-to-protein variations in color response are listed in “Protein-to-protein variation” on page 5. All proteins were tested at 1000 µg/mL. The average net color response for BSA was normalized to 1.00. The average net color response of the other proteins is expressed as a ratio to the response of BSA.

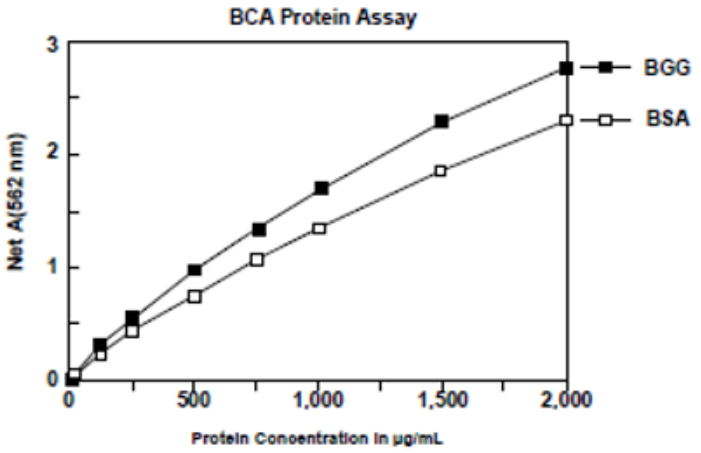

Figure 3

Typical color response curves for BSA and BGG using the standard cuvette protocol (37°C/30 minute incubation).

Protein-to-protein variation

Table 2 Protein-to-protein variation

Protein tested	Ratio [1] [2]
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.85
α-Chymotrypsinogen, bovine	1.14
Cytochrome C, horse heart	0.83
Gamma globulin, bovine	1.11
IgG, bovine	1.21
IgG, human	1.09
IgG, mouse	1.18
IgG, rabbit	1.12
IgG, sheep	1.17
Insulin, bovine pancreas	1.08
Myoglobin, horse heart	0.74
Ovalbumin	0.93
Transferrin, human	0.89
Average Ratio	**1.02**
Standard Deviation	**0.15**
Coefficient of Variation	**14.7%**

[1] Ratio = (avg. "test" net Abs.)/(avg. BSA net Abs.)
[2] Absorbance ratios (562 nm) for proteins relative to BSA.

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 3 Compatible substance concentrations

Salts/Buffers	Compatible Concentration
ACES, pH 7.8	25 mM
Ammonium sulfate	∅
Asparagine	1 mM
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
Borate (50 mM), pH 8.5 (Cat. No. 28384)	undiluted
B-PER™ Reagent (Cat. No. 78248)	undiluted
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)	1:8
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (Cat. No. 28388)	1:8
Cobalt chloride in TBS, pH 7.2	0.8 mM
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
Glycine	100 mM
Guanidine•HCl	4 M
HEPES, pH 7.5	100 mM
Imidazole,pH 7.0	50 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	undiluted
Sodium acetate, pH 4.8	200 mM
Sodium azide	0.2%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Sodium phosphate	100 mM
Tricine,pH 8.0	25 mM
Triethanolamine,pH 7.8	25 mM

Salts/Buffers	Compatible Concentration
Tris	250 mM
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)	1/3 diultion
Tris (25 mM), Glycine (192 mM), SDS (0.1%), pH 8.3 28378	undiluted
Zinc chloride in TBS, pH 7.2	10 mM

Detergents	Compatible Concentration
Brij™-35	5%
Brij™-56, Brij™-58	1%
CHAPS, CHAPSO	5.0%
Deoxycholic acid	0.05%
Lubrol™ PX	0.125%
Octyl β-glucoside	5%
Octyl β-thioglucopyranoside	5%
Nonidet P-40 (NP-40)	5%
SDS	5%
Span™-20	1%
Triton-X™-100, Triton-X™-114	1%
Triton-X™-305, Triton-X™-405	1%
Tween™-20, Tween™-60, Tween™-80	5%
Zwittergent™ 3-14	1%

Chelating Agents	Compatible Concentration
EDTA	10 mM
EGTA	∅
Sodium citrate	200 mM

Reducing and Thiol-containing Agents	Compatible Concentration
N-acetylglucosamine in PBS, pH 7.2	10 mM
Ascorbic acid	∅
Cysteine	∅
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
Glucose	10 mM
Melibiose	∅
2-Mercaptoethanol	0.01%
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	∅
PMSF	1 mM
Sodium Hydroxide	100 mM
Sodium vanadate (sodium salt), in PBS, pH 7.2	1 mM
Sucrose	40%
TLCK	0.1 mg/L
TPCK	0.1 mg/L
Urea	3 M

References

Smith, P.K. *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**:76-85.

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.

Revision history: Pub. No. MAN0029423 B.0

Revision	Date	Description
B.0	17 November 2023	Correcting concentrations in chemical compatibility table.
A.0	3 August 2023	New document for Pierce™ BCA Protein Assay Kit with Dilution-Free™ BSA Protein Standards.