Pierce™ BCA Protein Assay Kit

Catalog Numbers 23225, 23227 and A65453

Doc. Part No. 2161296 Pub. No. MAN0011430 Rev. C00

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 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 bicinchoninic acid (see reference 1). 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 (20–2000 µg/mL). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together.

The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA (see reference 2). Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual color-producing functional groups (see reference 2). Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknowns before the concentration of each unknown is determined based on the standard curve. 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; for example, a bovine gamma globulin (BGG) standard (see “Related products”) may be used when assaying immunoglobulin samples.

Two assay procedures are described in the user guide. The test tube procedure requires a larger volume (0.1 mL) of protein sample; however, it uses a sample to working reagent ratio of 1:20 (v/v), hence the effect of interfering substances is minimized. The microplate procedure allows the ease of using a microplate and requires a smaller volume (10–25 µL) of protein sample; however, because the sample to working reagent ratio is 1:8 (v/v), it offers less flexibility in overcoming interfering substance concentrations and obtaining low levels of detection.

Note: For peptide sample concentration measurements, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay or the Pierce™ Quantitative Colorimetric Peptide Assay Kit (see Related products).

Contents and storage

Item	Cat. No. 23225 (500 tubes or 5,000 microplate assays)	Cat. No. 23227 (250 tubes or 2,500 microplate assays)	Cat. No. A65453 (100 tubes or 1,000 microplate assays)	Storage
BCA Reagent A	2 x 500 mL	500 mL	100 mL	15–30 °C
BCA Reagent B	25 mL	25 mL	2.5 mL	
Albumin Standard Ampules	10 x 1 mL	10 x 1 mL	2 mL	

Workflow

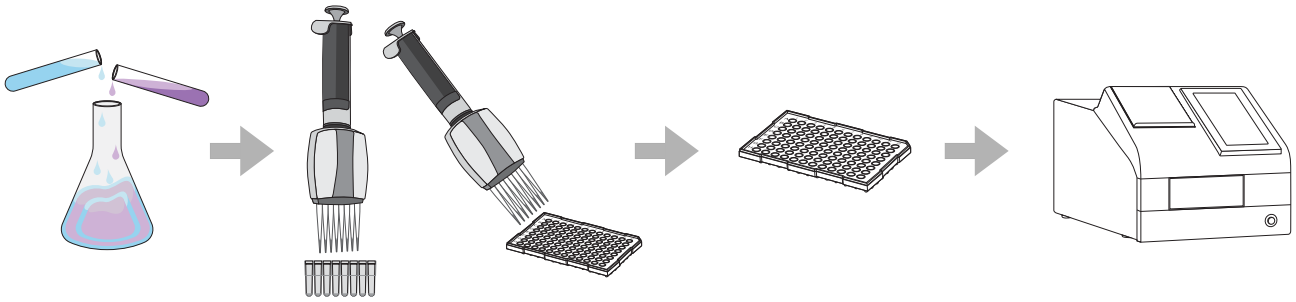

Figure 1 Procedure summary (microplate procedure, standard protocol)

This workflow diagram shows the Dilution-Free BSA Protein Standards (step 2, with multi-channel pipette hovering over top). These standards are not included in this kit but are included in Cat. No. A55864 and Cat. No. A55865. See Table 1 for further instructions on how to prepare BSA standards included in this kit.

Procedural guidelines

- For samples containing reducing or metal-chelating substances, we recommend using the Pierce™ Bradford Plus Protein Assay Kit (Catalog no. 23236) or the Pierce™ BCA Protein Assay Kit - Reducing Agent Compatible (Catalog no. 23250). For more information about eliminating interfering substances, go to **http://www.thermofisher.com**
- If reusing glassware, thoroughly clean, then rinse with ultrapure water before use with the kit.

Preparation of standards and working solutions

Prepare diluted albumin (BSA) standards

Dilute the BSA standard into several clean vials, preferably using the same diluent as the samples.

Use the following table as a guide to prepare a set of BSA standards. Each 1 mL of BSA standard is sufficient to prepare a set of diluted standards for either working range suggested in the table.

Table 1 BSA standard dilution scheme for the microplate procedure and the test tube procedure using the standard protocol (working range = 20–2,000 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 = Blank

Table 2 BSA standard dilution scheme for the test tube procedure using the enhanced protocol (working range = 5–250 µg/mL)

Vial	Volume of diluent	Volume and source of BSA	Final BSA concentration
A	700 µL	100 µL of stock	250 µg/mL
B	400 µL	400 µL of vial A dilution	125 µg/mL
C	450 µL	300 µL of vial B dilution	50 µg/mL
D	400 µL	400 µL of vial C dilution	25 µg/mL
E	400 µL	100 µL of vial D dilution	5 µg/mL
F	400 µL	0	0 = Blank

Prepare BCA working reagent (WR)

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

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

   Example: For the standard test-tube protocol with 3 unknowns and 2 replicates of each sample:

   (9 standards + 3 unknowns) × (2 replicates) × (2 mL) = 48 mL WR required

   Note: For the test tube protocol, 2.0 mL of WR is required for each sample, while only 200 µL of WR reagent is required for each sample in the microplate procedure.

2. Prepare WR by mixing 50 parts of BCA reagent A with 1 part of BCA reagent B (50:1, Reagent A:B). For the above example, combine 50 mL of reagent A with 1mL of reagent B.

   Note: When reagent B is first added to reagent A, turbidity is observed that quickly disappears on mixing to yield a clear, green WR. 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.

Test-tube procedure (sample to WR ratio = 1:20)

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

2. Add 2.0 mL of the WR to each tube and mix well.

3. Cover, then incubate the tubes. Use one of the following protocols:
   - Standard protocol: 37°C for 30 minutes (working range = 20–2,000 µg/mL)
   - Room temperature protocol: 15–30°C for 2 hours (working range = 20–2,000 µg/mL)
   - 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.
   - Use a water bath to heat tubes according to the standard (37°C incubation) or enhanced (60°C incubation) protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.

4. Equilibrate the tubes to room temperature.

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

   Note: Because the BCA assay does not reach a true end point, color development will continue even after equilibrating to room temperature. However, because the rate of color development is low at room temperature, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made in 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 standards 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.

Microplate procedure (sample to WR ratio = 1:8)

1. Pipette 25 µL of each standard or unknown sample replicate into a microplate well (working range = 20–2000 µg/mL). (For example, Thermo Scientific™ Pierce™ 96–Well Plates, Cat. 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, the working range is limited to 125–2,000 µg/mL.

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

3. Cover the plate and incubate at 37°C for 30 minutes.

4. Equilibrate the plate to room temperature. Measure the absorbance at or near 562 nm on a plate reader.

   Note:
   - Wavelengths from 540–590 nm have been used successfully with this method.
   - Plate readers use a shorter light path length than cuvette spectrophotometers, hence the microplate procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard test tube procedure. If higher 562 nm measurements are desired, increase the incubation time to 2 hours.
   - Increasing the incubation time or ratio of sample volume to WR increases the net 562 nm measurement for each well and lowers both the minimum detection level of the reagent and the working range of the assay. As long as all standards and unknowns are treated identically, such modifications are useful.

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 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 provides 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.

Response characteristics for different proteins

The commonly used total protein assay methods usually show some level of varying response toward different proteins. These differences relate to amino acid sequence, pI, structure and presence of specific side chains or prosthetic groups that can dramatically alter the protein's color response. Most protein assay methods use BSA or immunoglobulin (IgG) as the standard against which, the concentration of the protein in the sample is determined (Figure 2). However, if great accuracy is required, prepare the standard curve from a pure sample of the target protein.

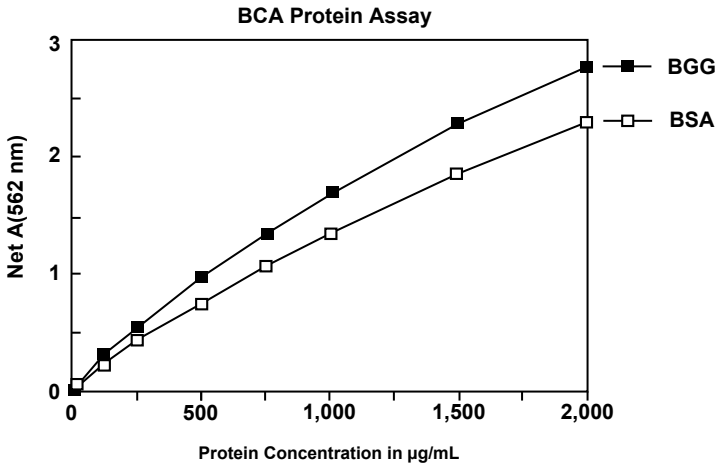

Figure 2 Typical color response curves for BSA and bovine gamma globulin (BGG) using the standard test tube protocol (37°C/30-minute incubation)

Table 3 Typical protein-to-protein variation in color response

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)[1]	
Protein	Ratio
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] Absorbance ratios (562 nm) for proteins relative to BSA. All proteins were tested at 1,000 µg/mL using the 30 minute/37ºC 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.

Related products

Cat. No.	Product
15041	**Pierce™ 96-Well Polystyrene Plates, Corner Notch,** 100 plates
15075	**Elisa Reagent Reservoir,** 200 units
15036	**Sealing Tape for 96-Well Plates,** 100 sheets.
A55863	**Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible,** 0.125-2 mg/mL, 7x1 mL
23209	**Albumin Standard Ampules, 2mg/mL,** 10 × 1mL ampules, containing bovine serum albumin (BSA)
23208	**Pierce™Bovine Serum Albumin Standard Pre-Diluted Set,** 7 × 3.5 mL
23212	**Pierce™Bovine Gamma Globulin Standard Ampules, 2 mg/mL,** 10 × 1mL ampules
A55860	**Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit,** 500 mL
23213	**Pierce™ Bovine Gamma Globulin Standard Pre-Diluted Set,** 7 × 3.5 mL
23246	**Pierce™ Detergent Compatible (Bradford)™ Assay Kit,** 300 test tube assays
23235	**Micro BCA™ Protein Assay Kit**, 500 mL
23290	**Pierce™ Quantitative Fluorometric Peptide Assays & Standards,** 500 assays
23275	**Pierce™ Quantitative Colorimetric Peptide Assays & Standards,** 500 assays
23236	**Pierce™ Bradford Plus Protein Assay Kit,** 950 mL
23215	**Compat-Able™ Protein Assay Preparation Reagent Kit,** 500 mL
23250	**Pierce™ BCA Protein Assay Kit - Reducing Agent Compatible,** 275 mL

Cited references

1. Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**:76-85.
2. Wiechelman, K., *et al.* (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Anal Biochem* **175**:231-7.
3. Kessler, R. and Fanestil, D. (1986). Interference by lipids in the determination of protein using bicinchoninic acid. *Anal Biochem* **159**:138-42.
4. Brown, R., *et al.* (1989). Protein measurement using bicinchoninic acid: elimination of interfering substances. *Anal Biochem* **180**:136-9.

Troubleshooting and FAQs

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- For troubleshooting information and FAQs for this product: **https://www.thermofisher.com/bcafaqs**
- To browse the database and search using keywords: **thermofisher.com/faqs**

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Revision history: Pub. No. MAN0011430 C00

Revision	Date	Description
C00	3 April 2024	Major changes done in the publication which included making changes to various sections in the publication and majority of the content in the document was revised.
B.0	30 January 2020	Moved troubleshooting content to thermofisher.com.
A.0	15 October 2015	Baseline for revision.