

Pierce™ BCA Protein Assay Kit

23225 23227

1296.9

Number	Description
23225	Pierce BCA Protein Assay Kit , sufficient reagents for 500 test-tube or 5000 microplate assays
23227	Pierce BCA Protein Assay Kit , sufficient reagents for 250 test-tube or 2500 microplate assays

Kit Contents:

BCA Reagent A, 1000mL (in Product No. 23225) or 500mL (in Product No. 23227), containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1M sodium hydroxide

BCA Reagent B, 25mL, containing 4% cupric sulfate

Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules, containing bovine serum albumin (BSA) at 2mg/mL in 0.9% saline and 0.05% sodium azide

Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Note: If either Reagent A or Reagent B precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or evidence of microbial contamination.

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Introduction

The Thermo Scientific™ Pierce™ BCA Protein Assay 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.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 562nm that is nearly linear with increasing protein concentrations over a broad working range (20-2000 $\mu\text{g/mL}$). 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.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.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 unknown(s) 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 Thermo Scientific Products) may be used when assaying immunoglobulin samples.

Two assay procedures are presented. Of these, the Test Tube Procedure requires a larger volume (0.1mL) of protein sample; however, because it uses a sample to working reagent ratio of 1:20 (v/v), the effect of interfering substances is minimized. The Microplate Procedure affords the sample handling ease of a microplate and requires a smaller volume (10-25µL) 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.

Preparation of Standards and Working Reagent (required for both assay procedures)

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as the sample(s). Each 1mL ampule of 2mg/mL Albumin 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 Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20-2,000µg/mL)			
<u>Vial</u>	<u>Volume of Diluent</u> (µL)	<u>Volume and Source of BSA</u> (µL)	<u>Final BSA Concentration</u> (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

Dilution Scheme for Enhanced Test Tube Protocol (Working Range = 5–250µg/mL)			
<u>Vial</u>	<u>Volume of Diluent</u> (µL)	<u>Volume and Source of BSA</u> (µL)	<u>Final BSA Concentration</u> (µg/mL)
A	700	100 of Stock	250
B	400	400 of vial A dilution	125
C	450	300 of vial B dilution	50
D	400	400 of vial C dilution	25
E	400	100 of vial D dilution	5
F	400	0	0 = Blank

B. Preparation of the BCA Working Reagent (WR)

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

$$(\# \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (\text{volume of WR per sample}) = \text{total volume WR required}$$

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

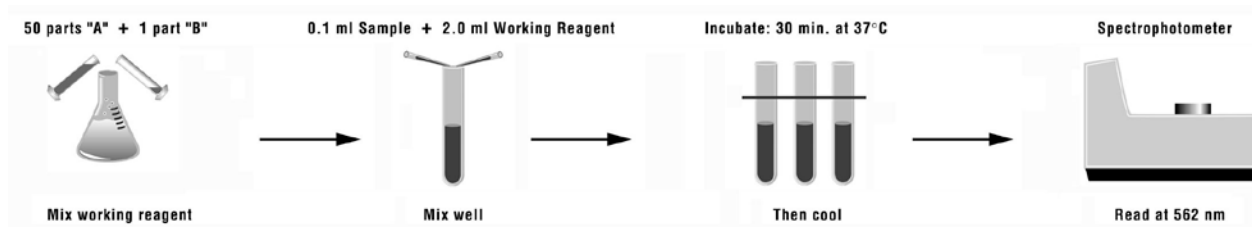
$$(9 \text{ standards} + 3 \text{ unknowns}) \times (2 \text{ replicates}) \times (2\text{mL}) = 48\text{mL WR required}$$

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

- 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 50mL of Reagent A with 1mL of Reagent B.

Note: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon 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 (RT).

Procedure Summary (Test-tube Procedure, Standard Protocol)



Test-tube Procedure (Sample to WR ratio = 1:20)

- Pipette 0.1mL of each standard and unknown sample replicate into an appropriately labeled test tube.
 - Add 2.0mL of the WR to each tube and mix well.
 - Cover and incubate tubes at selected temperature and time:
 - Standard Protocol: 37°C for 30 minutes (working range = 20-2000µg/mL)
 - RT Protocol: RT for 2 hours (working range = 20-2000µg/mL)
 - Enhanced Protocol: 60°C for 30 minutes (working range = 5-250µg/mL)
- Notes:**
- Increasing the incubation time or temperature increases the net 562nm 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 for either 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.
- Cool all tubes to RT.
 - With the spectrophotometer set to 562nm, zero the instrument on a cuvette filled only with water. Subsequently, 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, because the rate of color development is low at RT, no significant error will be introduced if the 562nm absorbance measurements of all tubes are made within 10 minutes of each other.
 - Subtract the average 562nm absorbance measurement of the Blank standard replicates from the 562nm absorbance measurement of all other individual standard and unknown sample replicates.
 - Prepare a standard curve by plotting the average Blank-corrected 562nm 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)

- Pipette 25µL of each standard or unknown sample replicate into a microplate well (working range = 20-2000µg/mL) (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).

Note: If sample size is limited, 10µL of each unknown sample and standard can be used (sample to WR ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2000µg/mL.
 - Add 200µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
 - Cover plate and incubate at 37°C for 30 minutes.
 - Cool plate to RT. Measure the absorbance at or near 562nm on a plate reader.
- Notes:**
- Wavelengths from 540-590nm have been used successfully with this method.
 - Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher 562nm measurements are desired, increase the incubation time to 2 hours.
 - Increasing the incubation time or ratio of sample volume to WR increases the net 562nm 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 may be useful.

5. Subtract the average 562nm absorbance measurement of the Blank standard replicates from the 562nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard vs. its concentration in $\mu\text{g/mL}$. 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.

Troubleshooting

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt or dilute sample Increase copper concentration in working reagent (e.g., use 50:2, Reagent A:B) Remove interfering substances from sample using Product No. 23215
Blank absorbance is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 562nm
Color of samples appears darker than expected	Protein concentration is too high	Dilute sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids ³ Remove interfering substances from sample using Product No. 23215
All tubes (including blank) are dark purple	Buffer contains a reducing agent	Dialyze or dilute sample
	Buffer contains a thiol	Remove interfering substances from sample using Product No. 23215
	Buffer contains biogenic amines (catecholamines)	
Need to measure color at a different wavelength	Spectrophotometer or plate reader does not have 562nm filter	Color may be measure at any wavelength between 540nm and 590nm, although the slope of standard curve and overall assay sensitivity will be reduced

A. Interfering substances

Certain substances are known to interfere with the BCA assay including those with reducing potential, chelating agents, and strong acids or bases. Because they are known to interfere with protein estimation at even minute concentrations, avoid the following substances as components of the sample buffer:

Ascorbic acid	EGTA	Iron	Impure sucrose
Catecholamines	Impure glycerol	Lipids	Tryptophan
Creatinine	Hydrogen peroxide	Melibiose	Tyrosine
Cysteine	Hydrazides	Phenol Red	Uric acid

Other substances interfere to a lesser extent with protein estimation using the BCA assay, and 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 Table 2 (see last page of Instructions). Substances were compatible at the indicated concentration in the Standard Test Tube Protocol if the error in protein concentration estimation caused by the presence of the substance was less than or equal to 10%. The substances were tested using WR prepared immediately before each experiment. Blank-corrected 562nm absorbance measurements (for a 1000 $\mu\text{g/mL}$ BSA standard + substance) were compared to the net 562nm measurements of the same standard prepared in 0.9% saline. Maximum compatible concentrations will be lower In the Microplate Procedure where the sample to WR ratio is 1:8 (v/v).

Furthermore, it is possible to have a substance additive affect such that even though a single component is present at a concentration below its listed compatibility, a sample buffer containing a combination of substances could interfere with the assay.

B. Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the Pierce BCA Protein Assay may be eliminated or overcome by one of several methods.

- Remove the interfering substance by dialysis or gel filtration.
- Dilute the sample until the substance no longer interferes. This strategy is effective only if the starting protein concentration is sufficient to remain in the working range of the assay upon dilution.
- Precipitate the proteins in the sample with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interfered is discarded and the protein pellet is easily solubilized in ultrapure water or directly in the alkaline BCA WR.⁴ A protocol detailing this procedure is available from our website. Alternatively, Product No. 23215 may be used (see Related Thermo Scientific Products).
- Increase the amount of copper in the WR (prepare WR as 50:2 or 50:3, Reagent A:B), which may eliminate interference by copper-chelating agents.

Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

Related Thermo Scientific Products

15041	Pierce 96-Well Plates, 100/pkg.
15075	Reagent Reservoirs, 200/pkg.
15036	Sealing Tape for 96-Well Plates, 100/pkg.
23209	Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules, containing bovine serum albumin (BSA)
23208	Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set, 7 × 3.5mL
23212	Bovine Gamma Globulin Standard, 2mg/mL, 10 × 1mL ampules
23213	Pre-Diluted Protein Assay Standards, (BGG) Set, 7 × 3.5mL aliquots
23235	Pierce Micro BCA Protein Assay Kit, working range of 0.5-20µg/mL
23236	Coomassie Plus™ (Bradford) Assay Kit, working range of 1-1500µg/mL
23215	Compat-Able™ Protein Assay Preparation Reagent Set
23250	Pierce BCA Protein Assay Kit—Reducing Agent Compatible

Additional Information

A. Please visit our website for additional information including the following items:

- Tech Tip #8: Eliminate interfering substances from samples for BCA Protein Assay

B. Alternative Total Protein Assay Reagents

If interference by a reducing substance or metal-chelating substance contained in the sample cannot be overcome, try the Thermo Scientific Coomassie Plus (Bradford) Assay Kit (Product No. 23236), which is less sensitive to such substances.

C. Cleaning and Re-using Glassware

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

D. 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, pI, structure and the presence of certain 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 protein in the sample is determined (Figure 1). However, if great accuracy is required, prepare the standard curve from a pure sample of the target protein.

Typical protein-to-protein variation in color response is listed in Table 3. All proteins were tested at 1000µg/mL using the 30-minute/37°C 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.

BCA Protein Assay

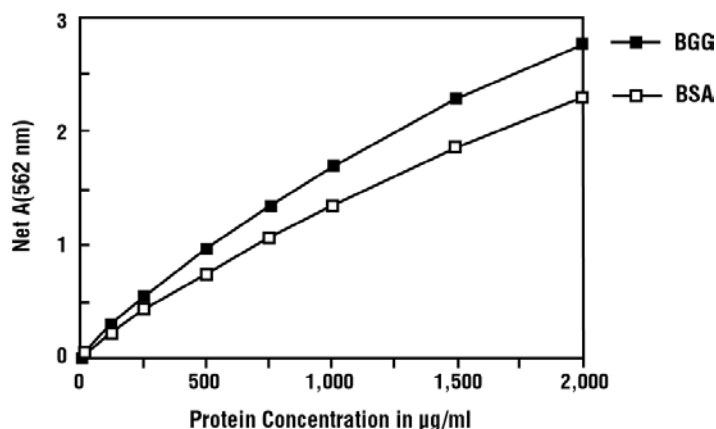


Figure 1: Typical color response curves for BSA and BGG using the Standard Test Tube Protocol (37°C/30-minute incubation).

Table 3. Protein-to-protein variation. Absorbance ratios (562nm) for proteins relative to BSA using the Standard Test Tube Protocol.

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)	
Protein Tested	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
	1.02
Standard Deviation	0.15
Coefficient of Variation	14.7%

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- 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.
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Table 2. Compatible substance concentrations in the Thermo Scientific Pierce BCA Protein Assay (see text for details).[§]

Substance	Compatible Concentration	Substance	Compatible Concentration
Salts/Buffers		Detergents**	
ACES, pH 7.8	25mM	Brij™-35	5.0%
Ammonium sulfate	1.5M	Brij-56, Brij-58	1.0%
Asparagine	1mM	CHAPS, CHAPSO	5.0%
Bicine, pH 8.4	20mM	Deoxycholic acid	5.0%
Bis-Tris, pH 6.5	33mM	Octyl β-glucoside	5.0%
Borate (50mM), pH 8.5 (# 28384)	undiluted	Nonidet P-40 (NP-40)	5.0%
B-PER™ Reagent (#78248)	undiluted	Octyl β-thioglucoopyranoside	5.0%
Calcium chloride in TBS, pH 7.2	10mM	SDS	5.0%
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4 (# 28382)	undiluted	Span™ 20	1.0%
Cesium bicarbonate	100mM	Triton™ X-100	5.0%
CHES, pH 9.0	100mM	Triton X-114, X-305, X-405	1.0%
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0 (# 28388)	1:8 dilution*	Tween™-20, Tween-60, Tween-80	5.0%
Na-Citrate (0.6M), MOPS (0.1M), pH 7.5 (#28386)	1:8 dilution*	Zwittergent™ 3-14	1.0%
Cobalt chloride in TBS, pH 7.2	0.8mM	Chelating agents	
EPPS, pH 8.0	100mM	EDTA	10mM
Ferric chloride in TBS, pH 7.2	10mM	EGTA	-----
Glycine•HCl, pH 2.8	100mM	Sodium citrate	200mM
Guanidine•HCl	4M	Reducing & Thiol-Containing Agents	
HEPES, pH 7.5	100mM	<i>N</i> -acetylglucosamine in PBS, pH 7.2	10mM
Imidazole, pH 7.0	50mM	Ascorbic acid	-----
MES, pH 6.1	100mM	Cysteine	-----
MES (0.1M), NaCl (0.9%), pH 4.7 (#28390)	undiluted	Dithioerythritol (DTE)	1mM
MOPS, pH 7.2	100mM	Dithiothreitol (DTT)	1mM
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted	Glucose	10mM
Nickel chloride in TBS, pH 7.2	10mM	Melibiose	-----
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2 (# 28372)	undiluted	2-Mercaptoethanol	0.01%
PIPES, pH 6.8	100mM	Potassium thiocyanate	3.0M
RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	undiluted	Thimerosal	0.01%
Sodium acetate, pH 4.8	200mM	Misc. Reagents & Solvents	
Sodium azide	0.2%	Acetone	10%
Sodium bicarbonate	100mM	Acetonitrile	10%
Sodium chloride	1M	Aprotinin	10mg/L
Sodium citrate, pH 4.8 or pH 6.4	200mM	DMF, DMSO	10%
Sodium phosphate	100mM	DMSO	10%
Tricine, pH 8.0	25mM	Ethanol	10%
Triethanolamine, pH 7.8	25mM	Glycerol (Fresh)	10%
Tris	250mM	Hydrazides	-----
TBS; Tris (25mM), NaCl (0.15M), pH 7.6 (# 28376)	undiluted	Hydrides (Na ₂ BH ₄ or NaCNBH ₃)	-----
Tris (25mM), Glycine (192mM), pH 8.0 (# 28380)	1:3 dilution*	Hydrochloric Acid	100mM
		Leupeptin	10mg/L

* Diluted with ultrapure water.

** Detergents were tested using high-purity Thermo Scientific Surfact-Amps Products, which have low peroxide content.

-- Dashed-line entry indicates that the material is incompatible with the assay.

[§] For a more extensive list of substances, download Tech Tip # 68: Protein assay compatibility table from our website. This Tech Tip includes compatible substances for all of our protein assays and enables easy comparisons.