

BCA™ Protein Assay Kit– Reducing Agent Compatible

23250

1745.0

Number	Description
23250	<p>BCA™ Protein Assay Kit–Reducing Agent Compatible, sufficient reagents to assay 250 samples or standards of 25 µl each</p> <p>Assay Working Range: 125-2,000 µg/ml</p> <p>Kit Contents:</p> <p>BCA™ Reagent A, 250 ml, contains sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartarate in 0.1 M sodium hydroxide</p> <p>BCA™ Reagent B, 25 ml, contains 4% cupric sulfate</p> <p>Compatibility Reagent, 10 × 20 mg</p> <p>Reconstitution Buffer, 15 ml</p> <p>Albumin Standard, 2 mg/ml, 10 × 1 ml ampules, contains bovine serum albumin at 2.0 mg/ml in 0.9% saline and 0.05% sodium azide</p> <p>Storage: Upon receipt store at room temperature.</p>

Introduction

The BCA™ Protein Assay Kit–Reducing Agent Compatible enables quantitation of total protein in samples while minimizing interference from disulfide reducing agents. The BCA™ Assay is based on the well-known reduction of Cu⁺² to Cu⁺¹ by protein in an alkaline medium and the highly sensitive and selective colorimetric detection of the cuprous cation using bicinchoninic acid (BCA).¹ Disulfide reducing agents, particularly dithiothreitol, 2-mercaptoethanol and TCEP are also capable of reducing Cu⁺² to Cu⁺¹. To minimize the affect of these copper reducers, a compatibility reagent that modifies disulfide reducing agents is added to the sample before adding the BCA™ Reagents.

This assay is also compatible with most ionic and non-ionic detergents in the presence of a disulfide reducing agent. Purification of membrane proteins presents unusual challenges to protein quantitation, as these proteins often require the presence of detergents and a disulfide reducing agent to maintain solubility and stability. The dual compatibility of this kit enables researchers to more accurately determine protein concentration for such samples.

Important Product Information

- BCA™ Protein Assay Kit–Reducing Agent Compatible is compatible with protein samples containing up to 5 mM DTT, 35 mM 2-mercaptoethanol or 10 mM TCEP.
- Certain substances interfere with the BCA™ Assay Kit–Reducing Agent Compatible, including chelating agents and strong acids or bases. Please see the Interfering Substances Section for more information.
- Always maintain the ionic strength of the sample buffer at ≤ 50 mM. When two or more interfering substances are present in the sample (e.g., DTT and SDS), the buffer ionic strength must be ≤ 20 mM.
- Standard curves generated in the range of 125 to 2,000 µg/ml using bovine serum albumin (BSA) or bovine gamma globulin (BGG) with and without disulfide reducing reagents produce < 5% slope variation (see Figure 1 in the Additional Information Section).
- If either BCA™ 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.

Warranty: Pierce products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sale for products used, handled and stored according to Pierce instructions. Pierce's sole liability for the product is limited to replacement of the product or refund of the purchase price. Pierce products are supplied for laboratory or manufacturing applications only. They are not intended for medicinal, diagnostic or therapeutic use. Pierce products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Pierce Biotechnology. Pierce strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce product, please contact Pierce or your local distributor.

Additional Materials Required

- 1.5 ml microcentrifuge tubes
- Pipettors and disposable pipette tips
- 37°C water bath

Procedure for Protein Quantitation using the Reducing Agent-Compatible BCA™ Assay

A. Blank and Standard Preparation

- **Standard Blank:** This Standard Blank does not contain protein. Prepare 200 µl of the same buffer as the unknown sample(s) without reducing agent.
- **Sample Blank:** This Sample Blank does not contain protein. Prepare 200 µl of the same buffer as the unknown sample with reducing agent at the same concentration as the sample.
- **Protein Standards:** Dilute the contents of one Albumin Standard (BSA) ampule into several microcentrifuge tubes, preferably using the same buffer as the unknown sample(s). Use the following table as a guide to prepare a set of standards with sufficient volume for three replications (assay range = 125-2,000 µg/ml):

<u>Vial</u>	<u>Diluent</u>	<u>BSA Source and</u>	<u>Concentration</u>
	<u>Volume (µl)</u>	<u>Volume (µl)</u>	<u>(µg/ml)</u>
A	0	500 of stock	2,000
B	125	375 of stock	1,500
C	200	200 of stock	1,000
D	200	200 of vial B	750
E	200	200 of vial C	500
F	200	200 of vial E	250
G	200	200 of vial F	125

B. Reagent Preparation

- Working Reconstitution Buffer: Dilute the Reconstitution Buffer 1:1 with ultrapure water. For protein samples with a buffer pH < 5, do not dilute the Reconstitution Buffer.
- Compatibility Reagent Stock Solution: Add 1 ml of either Working Reconstitution Buffer or Reconstitution Buffer to one tube of Compatibility Reagent and vortex at high speed for 30 seconds to dissolve. Store this solution for up to 8 hours at 4°C protected from light.
- BCA™ Working Reagent (WR): Use the following formula to determine the total volume of WR required:
 $(\# \text{ blanks} + \# \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (\text{volume of WR per sample}) = \text{total volume WR required.}$

Example: For three unknowns and two replicates of each sample: $(2 \text{ blanks} + 7 \text{ standards} + 3 \text{ unknowns}) \times (2 \text{ replicates}) \times (1 \text{ ml}) = 24 \text{ ml WR required.}$

To prepare the WR, mix 50 parts BCA™ Reagent A with one part of BCA™ Reagent B (50:1, Reagent A:B).

Note: When Reagent B is first added to Reagent A, the solution briefly appears turbid and yields a clear, green WR upon mixing.

C. Protein Quantitation

1. Pipette 25 µl of each replicate of standard, unknown sample and the Standard and Sample Blanks into 1.5 ml tubes.
2. Add 25 µl of Compatibility Reagent Stock Solution to each tube and vortex at low speed to mix. Proper mixing of samples with Compatibility Reagent Stock Solution is essential for assay accuracy.
3. Incubate tubes at 37°C for 15 minutes in a water bath. Using a forced-air incubator can introduce significant error from uneven heat transfer.
4. Add 1 ml of the WR to each tube and vortex to mix well.
5. Incubate tubes at 37°C for 30 minutes in a water bath.
6. Cool tubes at room temperature (RT) for 5-10 minutes.

7. With the spectrophotometer set to 562 nm, 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 slow at RT, no significant error is introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.
8. Subtract the average 562 nm absorbance value of the Standard Blank replicates from the 562 nm absorbance value of all standards.
9. Subtract the average 562 nm absorbance value of the Sample Blank replicates from the average 562 nm absorbance value of unknown sample replicates.
10. Prepare a standard curve by plotting the average blank-corrected 562 nm value for each BSA standard vs. its concentration (µg/ml). Use the standard curve to determine the protein concentration of each unknown sample.

Troubleshooting

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent at an incompatible concentration (see Table 1)	Dilute, dialyze or desalt the sample
		Increase copper concentration in Working Reagent (e.g., use 50:2, Reagent A:B)
Blank absorbance is OK, but standards and samples have less color than expected	Strong acid or alkaline buffer altered the Working Reagent pH	Dilute, dialyze or desalt sample
Color of samples including blank appear darker than expected	Sample contains a reducing agent at concentrations above the indicated compatible level	Dilute sample
	Sample contains biogenic amines (catecholamines)	Dilute, dialyze or desalt sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids ²

Interfering Substances

Certain substances interfere with the reducing agent-compatible BCA™ Assay, including those with reducing potential, chelating agents and strong acids or bases. Because they interfere with protein estimation at even minute concentrations, avoid the following substances as components of the sample buffer:

ascorbic acid	impure glycerol	lipids	tryptophan
catecholamines	hydrogen peroxide	melibiose	tyrosine
creatinine	hydrazides	phenol red	uric acid
EGTA	iron	impure sucrose	

Other substances interfere to a lesser extent, and they have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances are listed in Table 1. The listed concentrations are compatible with either 5 mM DTT or 35 mM 2-mercaptoethanol after modification with the Compatibility Reagent. Substances were considered compatible if the error in concentration estimation caused by the presence of the substance and the reducing agent was ≤ 10%. Blank-corrected 562 nm absorbance values for 1,000 µg/ml BSA and the Compatibility Reagent-modified substance were compared to the net 562 nm values of the same standard prepared in water.

Table 1. Compatible substance concentrations for the BCA™ Protein Assay Kit–Reducing Agent Compatible.

Detergents*		Buffers/Salts		Chelators	
Tween® -20	10%	Tris	50 mM	EDTA	20 mM
Triton® X-114	2%	HEPES, pH 7.5	200 mM	Sodium citrate	100 mM
Triton® X-100	10%	MES, pH 6.1	100 mM		
CHAPS	10%	Imidazole, pH 7.0	50 mM		
SDS	10%	Guanidine•HCl	2 M		
Octyl β-thioglucoopyranoside	10%	Urea	4 M		
Zwittergent® 3-14	2%	Sucrose	40%		

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

Additional Information

Standard curves generated in the range of 125 to 2,000 µg/ml using bovine serum albumin (BSA) with and without disulfide reducing reagents produce < 5% slope variation (Figure 1).

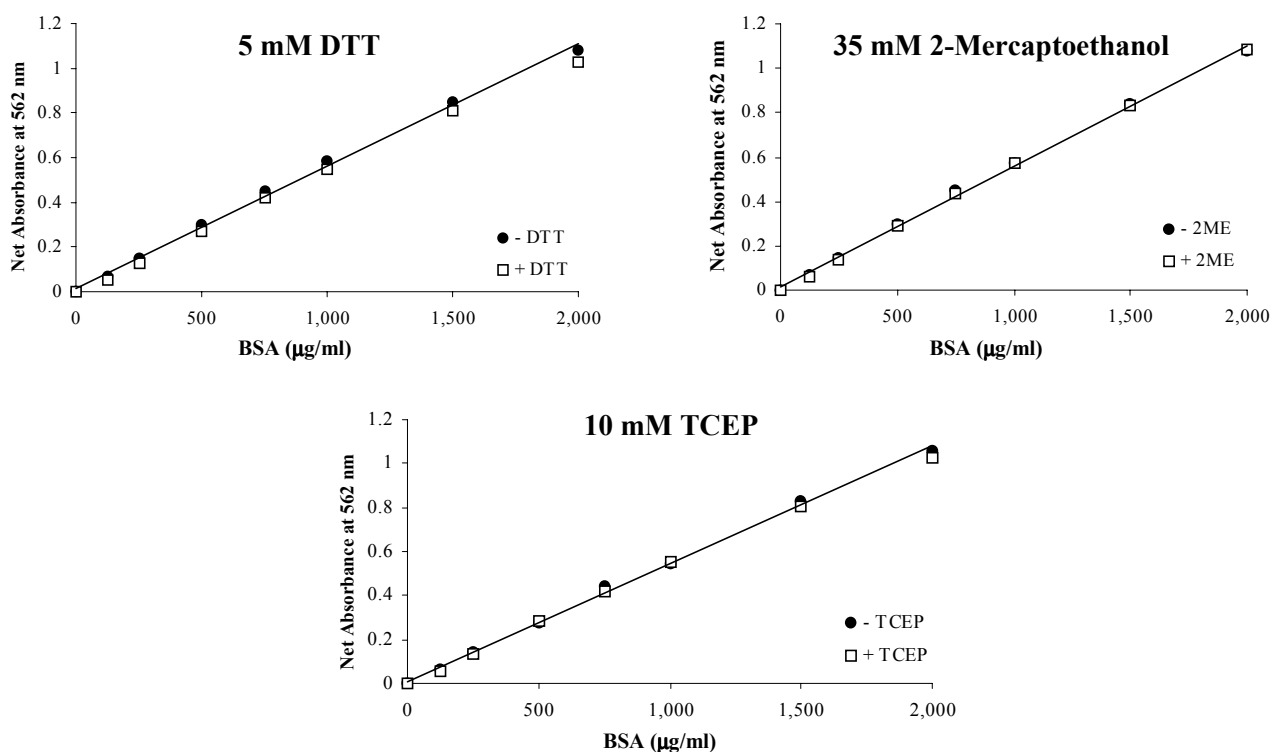


Figure 1. Standard curves generated using BSA with Compatibility Reagent and the presence and absence of 5 mM DTT, 35 mM 2-mercaptoethanol and 10 mM TCEP. Standard stock solutions were prepared in 20 mM Tris•HCl, 0.05% sodium azide, pH 8.0.

Related Pierce Products

- 23209 **Albumin Standard Ampules, 2 mg/ml, 10 × 1 ml ampules**
- 23208 **Pre-Diluted Protein Assay Standards: BSA Set, 7 × 3.5 ml ranging from 125 to 2,000 µg/ml**
- 23212 **Bovine Gamma Globulin Standard, 2 mg/ml, 10 × 1 ml ampules**
- 23213 **Pre-Diluted Protein Assay Standards, Bovine Gamma Globulin Fraction II (BGG) Set, 7 × 3.5 ml ranging from 125 to 2,000 µg/ml**
- 23221 **BCA™ Reagent A, 1,000 ml**
- 23223 **BCA™ Reagent A, 250 ml**
- 23224 **BCA™ Reagent B, 25 ml**
- 23235 **Micro BCA™ Protein Assay Kit, working range of 0.5-20 µg/ml**
- 23236 **Coomassie Plus - The Better Bradford™ Assay Kit, working range of 1-1,500 µg/ml**

References

1. Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150(1)**:76-85.
2. Kessler, R. and Fanestil, D. (1986). Interference by lipids in the determination of protein using bicinchoninic acid. *Anal. Biochem.* **159(1)**:138-42.

Patent pending on Reducing Agent Compatible BCA™ Technology

BCA™ Technology is protected by U.S. Patent # 4,839,295.

Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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