

# Pierce<sup>®</sup> BCA Protein Assay Kit – Reducing Agent Compatible

23250

1745.4

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

## Introduction

The Thermo Scientific Pierce 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  $\text{Cu}^{+2}$  to  $\text{Cu}^{+1}$  by protein in an alkaline medium and the highly sensitive and selective colorimetric detection of the cuprous cation using bicinchoninic acid (BCA).<sup>1</sup> Disulfide reducing agents, particularly dithiothreitol, 2-mercaptoethanol and TCEP are also capable of reducing  $\text{Cu}^{+2}$  to  $\text{Cu}^{+1}$ . 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

- The Pierce BCA Protein Assay Kit – Reducing Agent Compatible is compatible with protein samples containing up to 5mM DTT, 35mM 2-mercaptoethanol or 10mM TCEP.
- Certain substances interfere with the Pierce BCA Assay Kit – Reducing Agent Compatible, including chelating agents and strong acids or bases. Please see the Interfering Substances Section for more information.
- If the sample's pH is  $\leq 5.0$ , maintain the ionic strength of the sample buffer at  $\leq 50\text{mM}$ . When two or more interfering substances are present in the sample (e.g., DTT and SDS), the buffer ionic strength must be  $\leq 20\text{mM}$ .
- Standard curves generated in the range of 125 to 2,000µg/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.

## Additional Materials Required

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

## Procedure for the Reducing Agent-Compatible BCA Assay

### A. Blank and Standard Preparation

The best method to control for the effects of reducing agent is to add the same type and amount of reducing agent to each serially diluted protein standard as occurs in the samples. However, because this method is laborious and typically yields < 5% error compared to standards prepared without added reducing agent, the default procedure uses a simpler method involving two blanks. The Standard Blank has no reducing agent, allowing background absorbance of the standards to be determined; the Sample Blank contains reducing agent, allowing background absorbance of the samples to be determined.

- **Standard Blank:** This Standard Blank does not contain protein. Prepare 200µL of the same buffer as the unknown sample(s) without reducing agent.
- **Sample Blank:** This Sample Blank does not contain protein. Prepare 200µL 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 = 125-2,000µg/mL):

<u>Vial</u>	<u>Diluent</u> <u>Volume (µL)</u>	<u>BSA Source and</u> <u>Volume (µL)</u>	<u>Concentration</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 1mL 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 blanks + 7 standards + 3 unknowns) × (2 replicates) × (1mL) = 24mL 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 added to Reagent A, the solution appears turbid but yields a clear, green WR upon mixing.

### C. Protein Quantitation

1. Pipette 25µL of each replicate of standard, unknown sample and the Standard and Sample Blanks into 1.5mL tubes.
2. Add 25µL 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 1mL of the WR to each tube and vortex to mix well. Incubate tubes at 37°C for 30 minutes in a water bath.
5. Cool tubes at room temperature (RT) for 5-10 minutes.
6. 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 slow at RT, no significant error is introduced if the 562nm absorbance measurements of all tubes are made within 10 minutes of each other.
7. Subtract the average 562nm absorbance value of the Standard Blank replicates from the 562nm absorbance value of all standards.
8. Subtract the average 562nm absorbance value of the Sample Blank replicates from the average 562nm absorbance value of unknown sample replicates.
9. Prepare a standard curve by plotting the average blank-corrected 562nm value for each BSA standard vs. its concentration ( $\mu\text{g/mL}$ ). 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 contained reducing agent at concentration 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 <sup>2</sup>

## Interfering Substances

Certain substances interfere with the reducing agent-compatible BCA assay, including those with reducing potential, chelating agents and strong acids or bases. The following substances interfere even at very low concentrations: ascorbic acid, catecholamines, creatinine, impure glycerol, hydrogen peroxide, hydrazides, iron, certain lipids, melibiose, phenol red, impure sucrose, tryptophan, tyrosine, uric acid.

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 5mM DTT or 35mM 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  $\leq 10\%$ . Blank-corrected 562nm absorbance values for 1,000 $\mu\text{g/mL}$  BSA and the Compatibility Reagent-modified substance were compared to the net 562nm values of the same standard prepared in water.

**Table 1.** Compatible substance concentrations for the BCA Protein Assay Kit–Reducing Agent Compatible.<sup>§</sup>

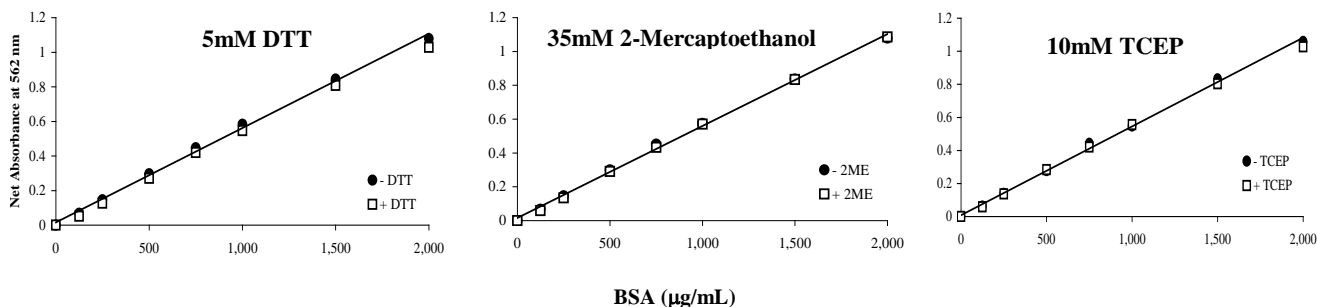
Detergents*		Buffers/Salts		Chelators	
Tween <sup>®</sup> -20	10%	Tris	50mM	EDTA	20mM
Triton <sup>®</sup> X-114	2%	HEPES, pH 7.5	200mM	EGTA	10mM
Triton X-100	10%	MES, pH 6.1	100mM	Sodium citrate	100mM
CHAPS	10%	Imidazole, pH 7.0	50mM		
SDS	10%	Guanidine•HCl	2M		
Octyl $\beta$ -thioglucopyranoside	10%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

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

<sup>§</sup>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.

## Additional Information

Standard curves generated in the range of 125 to 2,000 $\mu$ g/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 DTT, 2-mercaptoethanol and TCEP. Standard stock solutions were prepared in 20mM Tris•HCl, 0.05% sodium azide, pH 8.0.

## Related Thermo Scientific Products

23209	Albumin Standard Ampules, 2mg/mL, 10 $\times$ 1mL ampules
23252	Pierce Microplate BCA Protein Assay Kit – Reducing Agent Compatible, sufficient reagents for 1,000 microplate assays
23208	Pre-Diluted Protein Assay Standards: BSA Set, 7 $\times$ 3.5mL ranging from 125 to 2,000 $\mu$ g/mL
23212	Bovine Gamma Globulin Standard, 2mg/mL, 10 $\times$ 1mL ampules
23213	Pre-Diluted Protein Assay Standards, bovine gamma globulin fraction II (BGG) Set, 7 $\times$ 3.5mL ranging from 125 to 2,000 $\mu$ g/mL
23221	Pierce BCA Reagent A, 1,000mL
23223	Pierce BCA Reagent A, 250mL
23224	Pierce BCA Reagent B, 25mL
23235	Pierce Micro BCA Protein Assay Kit, working range of 0.5-20 $\mu$ g/mL
23236	Pierce Coomassie Plus (Bradford) Assay Kit, working range is 1-1,500 $\mu$ g/mL

## References

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

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