INSTRUCTIONS

Pierce® Microplate BCA Protein Assay Kit – Reducing Agent Compatible

23252

1997.3

Number	Description
23252	**Pierce Microplate BCA Protein Assay Kit – Reducing Agent Compatible,** sufficient reagents for 980 microplate assays
	Assay Working Range: 125-2,000µg/mL
	Kit Contents:
	BCA Reagent A, 250mL, contains sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartarate in 0.1M sodium hydroxide
	BCA Reagent B, 25mL, contains 4% cupric sulfate
	Compatibility Reagent No-Weigh Format, 9.3mg × 48 microtubes
	Reconstitution Buffer, 15mL
	Albumin Standard, 2 mg/ml, 10 × 1mL ampules, bovine serum albumin (BSA) in 0.9% NaCl and 0.05% sodium azide
	96-Well Microplates, 20 each
	Storage: Upon receipt store at room temperature.

Introduction

The Thermo Scientific Pierce Microplate BCA Protein Assay Kit – Reducing Agent Compatible enables quantitation of total protein in samples while minimizing interference from disulfide reducing agents. This kit allows simultaneous detection of up to 96 samples or standards and requires only 9 µl of protein sample, making it suitable when limited amounts of sample are available. The Pierce BCA Assay is based on the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium and the subsequent sensitive and selective colorimetric detection of the cuprous cation using bicinchoninic acid (BCA).[1] Disulfide reducing agents, particularly dithiothreitol (DTT), 2-mercaptoethanol and TCEP, also reduce copper. To minimize their reducing effect, a compatibility reagent that modifies disulfide reducing agents is added to the sample before performing the assay.

The 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, because 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

- This kit is compatible with protein samples containing up to 5mM DTT, 25mM 2-mercaptoethanol or 10mM TCEP.
- Certain substances interfere with the assay, including chelating reagents and strong acids or bases. Please see the Interfering Substances Section for more information.
- Always maintain the ionic strength of the sample buffer at ≤ 50mM. When two or more interfering substances are present in the sample (e.g., DTT and SDS), the buffer ionic strength must be ≤ 20mM.
- 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 < 7% slope variation (see Figure 1 in the Additional Information Section).
- If BCA Reagent A or B precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring the solution. Discard any kit reagent that is discolored or appears to have microbial contamination.

Additional Materials Required

- Pipettors and disposable pipette tips
- An incubator set at 37ºC

Procedure for the Pierce Microplate BCA Protein Assay Kit–Reducing Agent Compatible

A. Control and Standard Preparation

The best method for overcoming the reducing agent effects is to add the same type and amount of reducing agent to each serially diluted protein standard as present in the samples, but this method is laborious and typically yields < 7% error compared to standards prepared without added reducing agent. The procedure described in these instructions uses a simpler method involving two no-protein controls. These controls allow determination of the background absorbance of the standard and sample. The Standard Control contains no reducing agent, and the Sample Control contains reducing agent.

- **Standard Control:** This control does not contain protein. Prepare 200µL of the same buffer as the unknown sample(s) without reducing agent.
- **Sample Control:** This control 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 sample without the reducing agent. Use the following table as a guide to prepare a set of standards (assay range = 125-2,000µg/mL).

Vial	Diluent Volume (µL)	BSA Source and Volume (µL)	Concentration (µg/mL)
A	0	200 of stock	2,000
B	66	200 of stock	1,500
C	100	100 of vial A	1,000
D	100	100 of vial B	750
E	100	100 of vial C	500
F	100	100 of vial E	250
G	100	100 of vial F	125

Note: Do not discard any unused, undiluted BSA standard (2mg/mL). Store the BSA standard in a microcentrifuge tube at 4°C for future assays.

B. Reagent Preparation

Working Reconstitution Buffer	Dilute the Reconstitution Buffer 1:1 with ultrapure water; however, do not dilute the Reconstitution Buffer if the protein sample has a pH < 6, or if it contains EDTA or imidazole.
Compatibility Reagent Solution	Puncture the foil covering on the Compatibility Reagent tube with an empty pipette tip. Add 100µL of Working Reconstitution Buffer into the tube and dissolve by stirring at the bottom of the tube and pipetting up and down 15-20 times. Store this solution for up to 8 hours at 4°C protected from light. Used microtubes may be cut and discarded from the unused microtubes. Return the unused microtubes to pouch containing the desiccant pack. **Note**: For each sample, 4µL of Compatibility Reagent Solution is required.
BCA Working Reagent (WR)	Use the following formula to determine the total volume of WR required: (# controls + # standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required. Example: For three unknowns and two replicates of each sample: (2 controls + 7 standards + 3 unknowns) × (2 replicates) × (0.26 ml) = 6.24mL WR required. To prepare the WR, mix 50 parts BCA Reagent A with 1 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. Assay Protocol

- Precision pipetting is essential. For best results use 1-10µL pipettes. Completely evacuate the tip of any fluid. Small errors when pipetting account for large errors when measuring the absorbance.
- Add samples directly to the center of the well and avoid touching the sides of the well.
- The same tip may be used within the same group of samples. Use different tip for each sample when adding the Compatibility Reagent Solution.

1. If the protein sample has a pH < 5, dilute the sample 1:1 with Reconstitution Buffer.
2. Pipette 9µL of each replicate of Standard Control, Sample Control, standard and unknown sample to the center of the microplate well.
3. Add 4µL of Compatibility Reagent Solution to the sample in each well.
4. Cover plate and mix on a plate shaker at medium speed for one minute. Incubate plate at 37°C for 15 minutes.
5. Add 260µL of the WR to each well. Cover plate and mix on a plate shaker for one minute. If the samples contain detergents, cover the plate after mixing. Incubate plate at 37°C for 30 minutes.
6. Cool plate at room temperature (RT) for 5 minutes.
7. Use the Standard Control as the blank. Measure the absorbance of the standards, unknown samples, and Sample Controls at 562nm on a plate reader.

   Note: Because the BCA Assay does not reach a true end point, color development will continue even after cooling to RT. The absorbance increases at a rate of ~0.25% per minute at RT.
8. Subtract the average 562nm absorbance value of the Sample Control replicates from the 562nm absorbance value of all unknown sample replicates.
9. Prepare a standard curve by plotting the average blank-corrected 562nm value for each BSA standard vs. its concentration (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) curve produces more accurate results than a purely linear fit.

Troubleshooting

Problem	Possible Cause	Solution
No color in any wells	Sample contains a copper-chelating agent at an incompatible concentration (see Table 1)	Dilute, dialyze or desalt the sample
		Increase copper concentration in the 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 the 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 the sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference form 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. The following substances interfere even at low concentrations: ascorbic acid, catecholamines, creatinine, impure glycerol, hydrogen peroxide, hydrazides, iron, certain lipids, melibiose, phenol red, impure sucrose, tryptophan, tyrosine and 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 5mM DTT, 25mM 2-mercaptoethanol or 10mM TCEP 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 562nm absorbance values for 1,000µg/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 Pierce Microplate BCA Protein Assay Kit – Reducing Agent Compatible.

Detergents*		Buffers/Salts		Chelators	
Tween®-20	10%	Tris	35mM	EDTA	5mM
Triton® X-114	2%	HEPES, pH 7.5	200mM	EGTA	5mM
Triton X-100	7%	MES, pH 6.1	100mM	Sodium citrate	50mM
CHAPS	10%	Imidazole, pH 7.0	30mM	**Other**	
SDS	5%	Guanidine•HCl	1.5M	L-cysteine	2.5mM
Octyl β-thioglucopyranoside	7%	Urea	3M	Glutathione‡	10mM
Zwittergent® 3-14	2%	Sucrose	40%		

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

Additional Information

Standard curves generated ranging from 125 to 2,000µg/mL using bovine serum albumin (BSA) with and without disulfide reducing reagents produce < 7% slope variation (Figure 1).

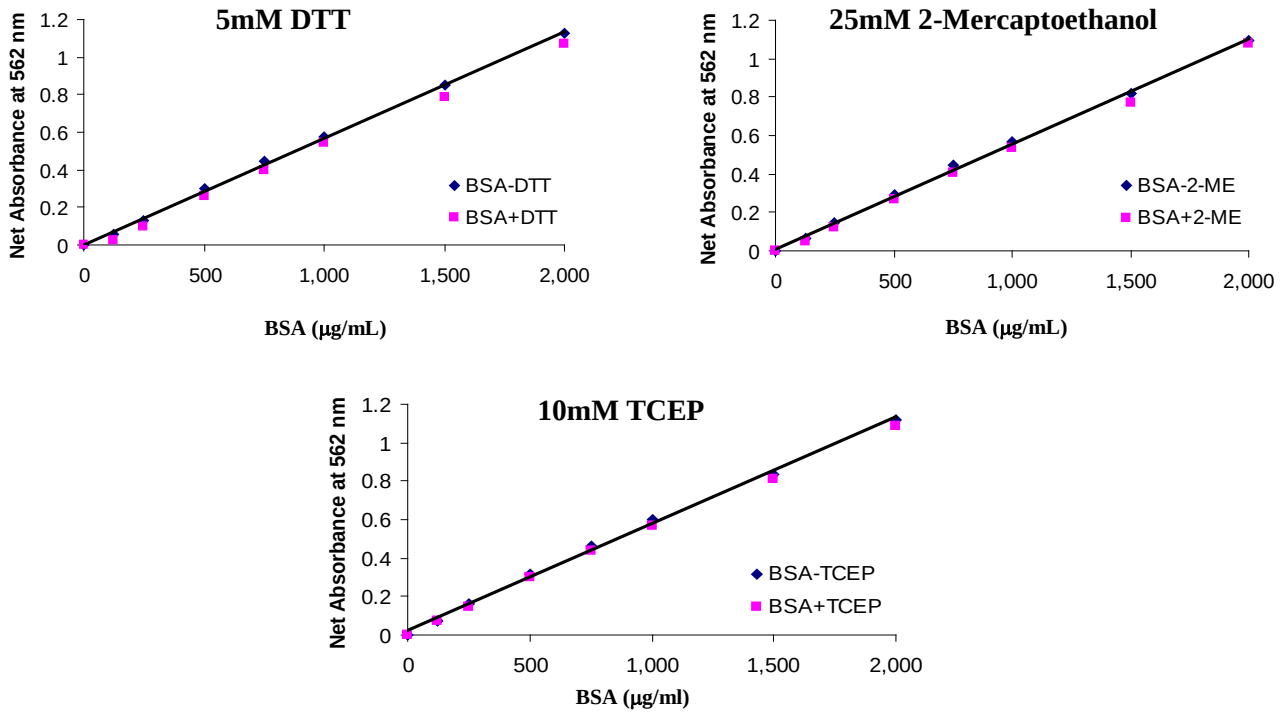

Figure 1. Standard curves generated using BSA with Compatibility Reagent in the presence and absence of 5mM DTT, 25mM 2-mercaptoethanol and 10mM TCEP. Standard stock solutions were prepared in 0.9% NaCl, 0.05% sodium azide.

Related Thermo Scientific Products

15045	**96-Well Microplates for Pierce BCA Reducing Agent Compatible Kit,** 5/pkg.
23209	**Albumin Standard Ampules, 2 mg/ml**, 10 × 1mL ampules
23208	**Pre-Diluted Protein Assay Standards: BSA Set**, 7 × 3.5mL ranging from 125 to 2,000µg/mL
23212	**Bovine Gamma Globulin Standard, 2 mg/ml**, 10 × 1mL ampules
23213	**Pre-Diluted Protein Assay Standards**, Bovine Gamma Globulin Fraction II (BGG) Set, 7 × 3.5mL ranging from 125 to 2,000µg/mL
23221	**BCA Reagent A**, 1,000mL
23223	**BCA Reagent A**, 250mL
23224	**BCA Reagent B**, 25mL
23250	**Pierce BCA Protein Assay Kit–Reducing Agent Compatible,** working range is 125-2,000µg/mL
23235	**Pierce Micro BCA Protein Assay Kit**, working range of 0.5-20µg/mL
23236	**Pierce Coomassie Plus (Bradford) Assay Kit**, working range is 1-1,500µg/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.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce