thermo scientific — USER GUIDE

Pierce™ Rapid Gold BCA Protein Assay Kit

Catalog Numbers A53225, A53226, and A53227

Doc. Part No. 2162702 **Pub. No.** MAN0017135 **Rev.** A.0

Product description

The Thermo Scientific™ Pierce™ Rapid Gold BCA Protein Assay Kit is a rapid protein assay that uses the same copper-chelating technology as the standard BCA assay with a unique chelator, which 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}). An adaptation of the traditional BCA assay, the Pierce™ Rapid Gold BCA Protein Assay Kit has been optimized to perform rapidly at room temperature and provide results within 5 minutes without the need for incubation at elevated temperatures. The protocol has been optimized for use in a microplate to reduce the required sample volume (≤ 20 µL) and read results quickly by use of a plate reader. Similar BSA standard curves are obtained using the Pierce™ Rapid Gold BCA Protein Assay Kit for 5 minutes at room temperature as compared to the traditional BCA assay for 30 minutes at 37°C. (Figure 1). While optimized for a microplate protocol, the assay can also be used with test tubes. For consistency of results, the test-tube method may require a stopping step using 1 N HCl if more than 15 samples are to be assayed (see below for detailed procedure).

While the traditional BCA assay produces a purple reaction product, the Pierce™ Rapid Gold BCA Protein Assay Kit produces an orange-gold-colored reaction product, which is formed by the chelation of 2 molecules of the chelator with 1 cuprous ion. This water-soluble complex exhibits a strong absorbance at 480 nm that is very linear with increasing protein concentrations over a broad working range of 20-2000 µg/mL. Protein concentrations are generally 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 an accurate concentration 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 (Product No. 23212) may be used when assaying immunoglobulin samples.

Note: For peptide sample concentration measurements, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay (Product No. 23290) or Pierce™ Quantitative Colorimetric Peptide Assay (Product No. 23275).

Contents

Product	Cat. No.	Contents	Storage
Pierce™ Rapid Gold BCA Protein Assay Kit	A53225	**500 mL kit sufficient for 2,400 microplate assays or 250 test-tube assays** **Contents:** **Rapid Gold BCA Reagent A containing a proprietary copper chelator**, 2 × 250 mL **Rapid Gold BCA Reagent B**, 10 mL, contains cupric sulfate **Albumin Standard Ampules**, 2 mg/mL, 10 × 1 mL ampules, contain bovine serum albumin (BSA) at 2 mg/mL in 0.9% saline and 0.05% sodium azide	Store at 4°C.
	A53226	**250 mL kit sufficient for 1,200 microplate assays or 125 test-tube assays** **Contents:** **Rapid Gold BCA Reagent A containing a proprietary copper chelator**, 250 mL **Rapid Gold BCA Reagent B**, 10 mL, contains cupric sulfate **Albumin Standard Ampules**, 2 mg/mL, 10 × 1 mL ampules, contain bovine serum albumin (BSA) at 2 mg/mL in 0.9% saline and 0.05% sodium azide	
	A53227	**Trial size, 20 mL kit, sufficient for 96 microplate assays or 10 test-tube assays** **Contents:** **Rapid Gold BCA Reagent A containing a proprietary copper chelator**, 20 mL **Rapid Gold BCA Reagent B**, 1 mL **Note:** Trial size kit does not include ampule standards.	

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Prepare Diluted Albumin (BSA) Standards (required for both assay procedures)

Use Table 1 as a guide to prepare a set of protein standards.

Use care when reusing glassware. All glassware must be cleaned and given a thorough final rinse with ultrapure water.

Table 1 Dilution scheme for microplate procedure (working range = 20-2,000 µg/mL)

Vial	Volume of diluent (µL)	Volume and source of BSA (µL)	Final BSA concentration (µg/mL)
A	0	300 (Stock)	2,000
B	62.5	187.5 (Stock)	1,500
C	100	100 (Stock)	1,000
D	100	100 (vial B dilution)	750
E	100	100 (vial C dilution)	500
F	100	100 (vial E dilution)	250
G	100	100 (vial F dilution)	125
H	160	40 (vial G dilution)	25
I	200	0	0

Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as the sample(s). Each 1 mL ampule of 2 mg/mL Albumin Standard is sufficient to prepare a set of diluted standards for the working range suggested in Table 1.

There will be sufficient volume for 3 replications of each diluted standard.

Prepare Pierce Rapid Gold BCA Working Reagent (WR) (required for both assay procedures)

Use care when reusing glassware. All glassware must be cleaned and given a thorough final rinse with ultrapure water.

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

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

   For example, for the standard microplate procedure with 3 unknowns and 2 replicates of each sample:

   (8 standards + 5 unknowns) × (2 replicates) × (0.2 mL) = 7.8 mL WR required (prepare 10 mL of WR to ensure enough WR is available for the assay)

   Note: A volume of 200 µL of WR is required for each sample in the microplate procedure.
2. Prepare WR by mixing 50 parts Rapid Gold BCA Reagent A with 1 part Rapid Gold BCA Reagent B (50:1, Reagent A:B). For the above example, combine 10 mL of Reagent A with 0.2 mL of Reagent B.

   Note: When Rapid Gold BCA Reagent B is first added to Rapid Gold BCA Reagent A, a pale blue precipitate may be observed, but, upon vortexing or mixing for < 5 seconds, the precipitate should dissolve to yield a clear, green solution.

   After making the WR, it is recommended to use the fresh solution. Upon standing at room temperature, it is normal to see a slight color shift to darker green for the WR. The standard curve is NOT affected when the signal is subtracted from the blank signal as long as the WR is used within 1.5 hours.

Perform microplate procedure (sample to WR ratio 1:10)

1. Pipette 20 µL of each standard or unknown sample replicate into a microplate well (working range = 20-2,000 µg/mL) (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product 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).
2. Add 200 µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Incubate at room temperature for 5 minutes.
4. Measure the absorbance at or near 480 nm on a plate reader.

   Note: Because the assay acts quickly, the plate should be read within 20-30 minutes to ensure color development of the top standard (2 mg/mL BSA) is not out of instrument linearity. In the event plate reading must be done after 30 minutes, the reaction can be stopped by using 50 µL of 1 N HCl. Using a stop solution allows the plate to be read up to 1 hour post-incubation with < 7% difference in results (see Figure 2).
5. Subtract the average 480 nm absorbance measurement of the blank standard replicates from the 480 nm measurements of all other individual standard and unknown sample replicates.

   Note: The absorbance reading of the blank (water/buffer + WR) at 480 nm is slightly higher than what is typically seen with the BCA assay at 562 nm. Therefore, it is important that the blank absorbance value be subtracted from the absorbance value of the standards and samples to obtain the correct concentration of the unknown samples.
6. Prepare a standard curve by plotting the average blank-corrected 480 nm measurement for each BSA standard versus its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Perform test tube procedure (standard protocol with sample to WR ratio 1:25)

1. Pipette 80 µL of each standard and unknown sample replicate into appropriately labeled test tubes. Include a water blank.
2. Add 2 mL of the WR to each tube and mix well (blank = water + WR).
3. Incubate tubes for 5 minutes at room temperature.
4. With the spectrophotometer set to 480 nm, zero the instrument using the blank. Subsequently, measure the absorbance of all the samples within 10 minutes.

   Note: This assay reacts more rapidly than the traditional BCA assay and, like the traditional BCA assay, it does not reach a true end point. Color development will continue at a much faster rate than traditional BCA. If your sample numbers (including standards) are < 15, you can measure the absorbance without having to stop the reaction. If you have > 15 samples, it is recommended to stop the assay by addition of 500 µL of 1 N HCl after the 5 minute incubation. The use of this stop reagent will significantly slow down the assay allowing for the tubes to be read within 30 minutes with no error between measurements.

5. Subtract the average 480 nm absorbance measurement of the blank standard replicates from the 480 nm absorbance measurement of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 480 nm measurement for each BSA standard versus 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 linear curve will provide very accurate results.

Related products

Product	Cat. no.
Pierce™ 96-Well Plates	15041
Reagent Reservoirs	15075
Sealing Tape for 96-Well Plates	15036
Albumin Standard Ampules, 2 mg/mL	23209
Pre-Diluted Protein Assay Standards, Bovine Serum Albumin (BSA) Set	23208
Bovine Gamma Globulin Standard, 2 mg/mL	23212
Pre-Diluted Protein Assay Standards, Bovine Gamma Globulin (BGG) Set	23213
Pierce™ Detergent Compatible Bradford Assay Kit	23246
Pierce™ Micro BCA Protein Assay Kit	23235
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Pierce™ Quantitative Colorimetric Peptide Assay	23275
Coomassie Plus™ (Bradford) Assay Kit	23236
Pierce™ BCA Protein Assay Kit-Reducing Agent Compatible	23250

Troubleshooting

Observation	Possible cause	Recommended action
No color in tubes.	Sample contained a copper chelating agent.	Dialyze, desalt, or dilute sample.
		Increase copper concentration in WR (working reagent) (e.g., use 50:2 versus typical 50:1 ratio)
Standards and samples show less color than expected. Blank absorbance is correct.	Strong acid or alkaline buffers altered WR pH.	Dialyze, desalt, or dilute sample.
	Color was measured at the wrong wavelength.	Measure the absorbance at 480 nm.
Sample color is darker than expected.	Protein concentration was too high.	Dilute sample.
All tubes (including blank) are orange-gold in color.	Buffer contained a reducing agent.	Dialyze or dilute sample.
	Buffer contained a thiol.	Dialyze or dilute sample.
	Buffer contained biogenic amines (catecholamines).	Dialyze or dilute sample.
Cannot measure color at a specific wavelength.	Spectrophotometer or plate reader did not have 480 nm filter.	Color may be measured at any wavelength between 460-500 nm, but the standard curve slope and overall assay sensitivity will be slightly reduced (<10%).

Additional information

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:

Components to avoid in 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 can interfere with protein estimation using the BCA assay, but these have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the microplate protocol are listed in Table 3. Some compatible reagents (listed below) in the microplate format for traditional BCA assays were found to be incompatible with the Rapid Gold BCA assay. The substances were tested using WR prepared immediately before each experiment. Blank-corrected 480 nm absorbance measurements (for a 1000 µg/mL BSA standard + substance) were compared to the net 480 nm measurements of the same standard prepared in ultrapure water. Maximum compatible concentrations will be higher in the test-tube procedure where the sample to WR ratio is 1:25 (v/v).

Reagent compatibility differences between traditional BCA and Rapid Gold BCA protein assays in a microplate			
1% SPAN 20 (compatible in Rapid Gold BCA assay at 0.5%)	Y-PER™ reagent (neet) (not compatible with Rapid Gold BCA assay)	50 mM imidazole (compatible in Rapid Gold BCA assay at 12.5 mM)	Sodium carbonate-sodium bicarbonate buffer, pH 9.4 (Product No. 28382) (compatible with Rapid Gold BCA assay at 1:2 dilution)

Furthermore, it is possible to have a substance-additive effect 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.

Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the Rapid Gold BCA Protein Assay may be eliminated 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.
- A protocol detailing this procedure is available from our website. Alternatively, Product No. 23215 may be used (see related products).
- Increase the amount of copper in the WR (prepare WR as 50:2 or 50:3, Reagent A:B) to aid in eliminating interference caused by copper-chelating agents.

Note: For greatest accuracy, the protein standards must be treated identically to the sample(s). The Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit (Product No. 23246) is an alternative related product compatible with a wide range of detergents.

Alternative total protein assay reagents

If interference by a reducing substance or metal-chelating substance contained in the sample cannot be overcome, try the Pierce™ Coomassie Plus (Bradford) Assay Kit (Product No. 23236) or Pierce™ 660nm Protein Assay Kit (Product No. 22662), which is less sensitive to such substances.

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. 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 2. All proteins were tested at 1,000 µg/mL using the microplate protocol for 5 minutes at room temperature. 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.

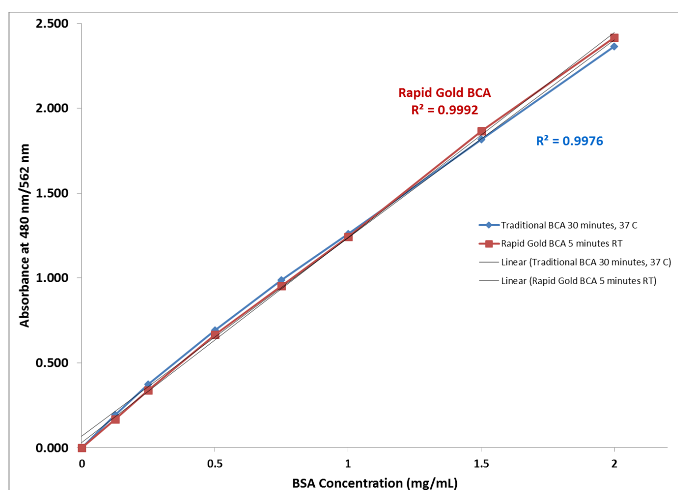

Fig. 1 Standard curves obtained using BSA with the Pierce Rapid Gold BCA assay versus the traditional BCA assay. WR for both was prepared by mixing Reagent A and Reagent B in a 50:1 ratio. Sample volumes and conditions for the Pierce Rapid Gold BCA assay and the traditional BCA assay were 20 µL at room temperature for 5 minutes, and 25 µL at 37°C for 30 minutes, respectively.

Table 2 Protein-to-protein variation. Absorbance ratios (480 nm) for proteins relative to BSA using the microplate protocol.

Protein Tested	Ratio (avg. test net absorbance) / (avg. BSA net absorbance)
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	1.01
α-Chymotrypsinogen, bovine	1.33
Cytochrome C, horse heart	1.13
Gamma globulin, bovine	1.42
IgG, bovine	1.44
IgG, human	1.32
IgG, mouse	1.21
IgG, rabbit	1.34
IgG, sheep	1.06
Insulin, bovine pancreas	1.00
Myoglobin, horse heart	0.85
Ovalbumin	1.09
Transferrin, human	1.00
Mean	**1.16**
Standard Deviation	**0.19**
Coefficient of Variation	**16.1%**

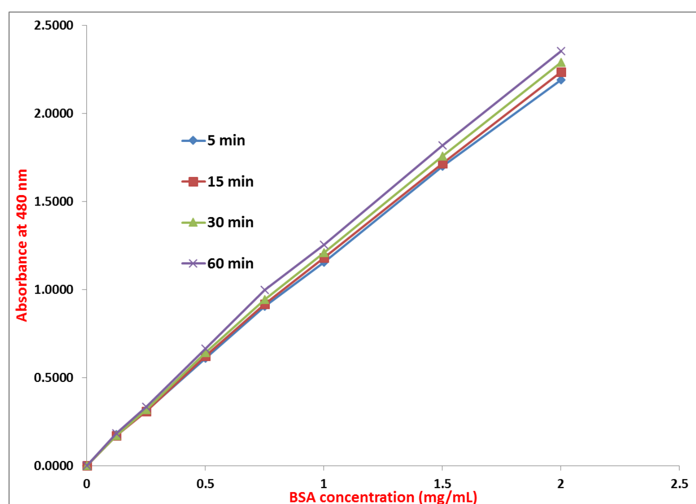

Fig. 2 Absorbance at 480 nm read up to 1 hour after incubation after 1 N HCl was used to stop the reaction.

Table 3 Assay compatibility for various substances.

Substance	Compatible Concentration	Substance	Compatible Concentration
Salts/Buffers		**Detergents****	
ACES, pH 7.8	25 mM	Brij™-35	5.0%
Bicine, pH 8.4	20 mM	Brij™-58	1.0%
Bis-Tris, pH 6.5	33 mM	CHAPS, CHAPSO	5.0%
Borate (50 mM), pH 8.5 (Product No. 28384)	undiluted	Na Deoxycholate (DOC)	5.0%
B-PER™ Reagent (Product No. 78248)	undiluted	Octyl ß-glucoside	5.0%
Calcium chloride in TBS, pH 7.2	10 mM	SDS	5.0%
Na-Carbonate/Na-Bicarbonate (0.2 M), pH 9.4 (Product No. 28382)	1:2 dilution*	Span™-20	0.5%
Cesium bicarbonate	100 mM	Triton-X™-100	5.0%
CHES, pH 9.0	100 mM	Triton-X™-114, Triton-X™-305, Triton-X™-405	1.0%
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (Product No. 28388)	1:8 dilution*	Tween™-20, Tween™-60, Tween™-80	5.0%
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (Product No. 28386)	1:8 dilution*	Zwittergent™ 3-14	1.0%
Cobalt chloride in TBS, pH 7.2	0.8 mM	**Chelating Agents**	
EPPS, pH 8.0	100 mM	EDTA	10 mM
Ferric chloride in TBS, pH 7.2	10 mM	Sodium citrate	200 mM
Glycine•HCl, pH 2.8	100 mM	**Reducing and Thiol-containing Agents**	
Guanidine•HCl	4 M	N-acetylglucosamine in PBS, pH 7.2	10 mM
HEPES, pH 7.5	100 mM	Glucose	10 mM
Imidazole, pH 7.0	12.5 mM		
MES, pH 6.1	100 mM		
MES (0.1M), NaCl (0.9%), pH 4.7 (Product No. 28390)	undiluted	**Misc. Reagents and Solvents**	
MOPS, pH 7.2	100 mM	Acetone	10%
Modified Dulbecco's PBS, pH 7.4 (Product No. 28374)	undiluted	Acetonitrile	10%
Nickel chloride in TBS, pH 7.2	10 mM	Aprotinin	10 mg/L
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (# 28372)	undiluted	DMF, DMSO	10%
PIPES, pH 6.8	100 mM	DMSO	10%
RIPA lysis buffer: 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	undiluted	Ethanol	10%
Sodium acetate, pH 4.8	200 mM	Glycerol (fresh)	10%
Sodium azide	0.2%	Hydrochloric acid	100 mM
Sodium bicarbonate	100 mM	Leupeptin	10 mg/L
Sodium chloride	1 M	N/A	
Sodium citrate, pH 4.8 or pH 6.4	200 mM		
Sodium phosphate	100 mM		
Tricine, pH 8.0	25 mM		
Triethanolamine, pH 7.8	25 mM		
Tris	250 mM		
TBS: Tris (25 mM), NaCl (0.15 M), pH 7.6 (Product No. 28376)	undiluted		
Tris (25 mM), Glycine (192 mM), pH 8.0 (Product No. 28380)	1:2 dilution*		

*Diluted with ultrapure water

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

Note: 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.

Product references

Adilakshami, T. and Laine, R.O. (2002). Ribosomal protein S25 mRNA partners with MTF-1 and La to provide a p53-mediated mechanism for survival or death. *J Biol Chem* **277:**4147-51.

Fischer, T., *et al.* (1999). Clathrin-coated vesicles bearing GAIP possess GTPase-activating protein activity in vitro. *Proc Nat Acad Sci* **96:**6722-7.

Prozialeck, W.C., *et al.* (2002). Chlamydia trachomatis disrupts N-cadherin-dependent cell-cell junctions and sequester b-catenin in human cervical epithelial cells. *Infection and Immunity* **70:**2605-13.

Roberts, K.P., *et al.* (2002). A comparative analysis of expression and processing of the rat epididymal fluid and sperm-bound forms of proteins D and E. *Biology of Reproduction* **67:**525-33.

Manufacturer: Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.