Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit

Catalog Numbers A55860, A55861, and A55862

Pub. No. MAN0029413 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Pierce™ Dilution-Free™ 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™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit has been optimized to perform rapidly at room temperature and provide results within 5 minutes.

This protein assay has been developed for use across a broad range (0.02–10 mg/mL), enabling most samples to be used neat (undiluted) and eliminating the guesswork and dilution steps that accompany traditional protein quantitation methods. The required sample volume is only 10 µL, allowing the user to conserve precious sample.

The Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit provides a set of pre-diluted bovine serum albumin (BSA) protein standards in a convenient eight-channel tubestrip that is compatible with a multi-channel pipette allowing all protein standards and blank solution to be transferred to a microplate simultaneously. While optimized for a microplate protocol, the assay can also be used with cuvettes.

Although the traditional BCA assay produces a purple reaction product, the Pierce™ Dilution-Free™ 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 linear with increasing protein concentrations over a broad working range of 20–10,000 µg/mL.

Workflow

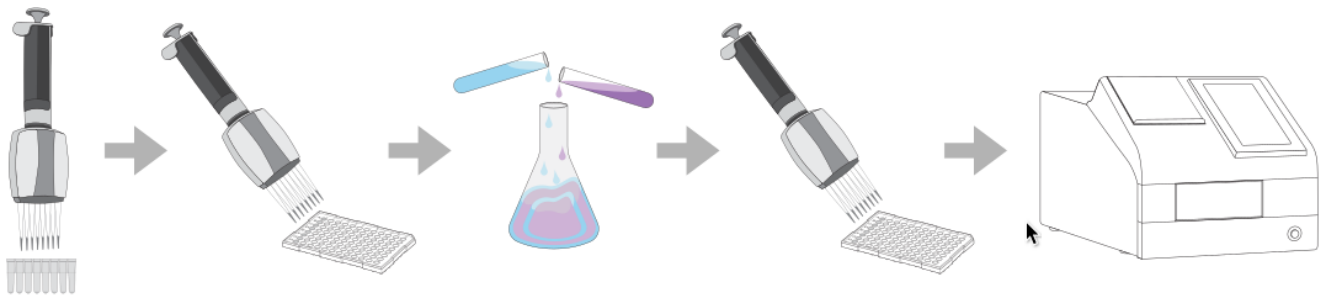

Figure 1 Protocol summary

Contents and storage

Table 1 Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit

Cat. No.	Contents	Storage
A55860	**500 mL kit sufficient for 2,400 microplate assays or 250 cuvette assays** **Contents:** **Dilution-Free™Rapid Gold BCA Reagent A**, 2 × 250 mL **Dilution-Free™ Rapid Bold BCA Reagent B**, 10 mL **Albumin Standards**, 2 × 1 mL, 8-channel tubestrip containing bovine serum albumin (BSA) at concentrations referenced below in 0.9% saline and 0.05% sodium azide	4°C
A55861	**250 mL kit sufficient for 1,200 microplate assays or 125 cuvette assays** **Contents:** **Dilution-Free™Rapid Gold BCA Reagent A**, 250 mL **Dilution-Free™Rapid Gold BCA Reagent B**, 10 mL **Albumin Standards**, 2 × 1 mL, 8-channel tubestrip containing bovine serum albumin (BSA) at concentrations referenced below in 0.9% saline and 0.05% sodium azide	
A55862	**20 mL kit (Trial size), sufficient for 96 microplate assays or 10 cuvette assays** **Contents:** **Dilution-Free™Rapid Gold BCA Reagent A**, 20 mL **Dilution-Free™Rapid Gold BCA Reagent B**, 1 mL **Albumin Standards**, 1 x 1 mL, 8-channel tubestrip containing bovine serum albumin (BSA) at concentrations referenced below in 0.9% saline and 0.05% sodium azide	

Prepare BSA standards

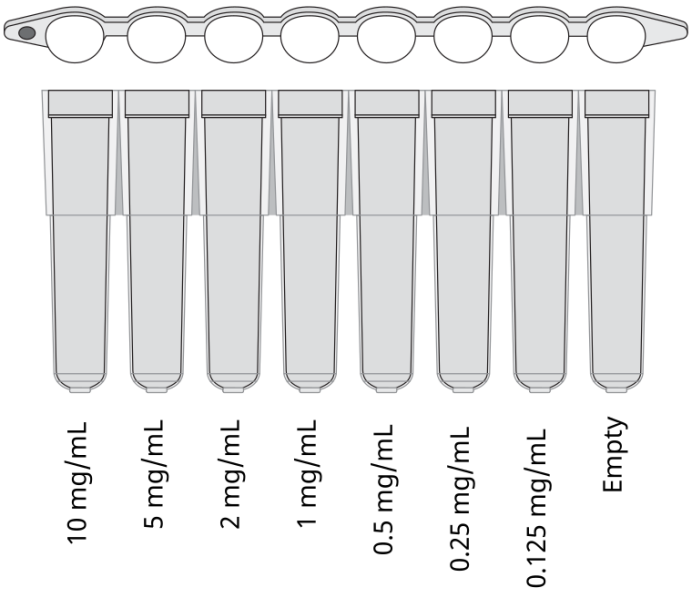

Figure 2 Pierce™ Dilution-Free™ BSA protein standards tubestrip format indicating the BSA protein concentration of each tube

Each tube of the tubestrip contains 1 mL of corresponding BSA concentration in a format compatible with a multichannel pipette. One tube is supplied empty allowing for the addition of a blank solution.

1. When ready to use, carefully remove foil from the tubestrip.
2. After foil is removed, the provided strip cap will be used to reseal the tubes. To keep the strip cap oriented there is a small hole at one end of the cap strip which should be aligned with the 10 mg/mL standard. The tubestrip has a single label on the tube containing the 10 mg/mL solution.
3. Push the cap strip firmly onto the trubestrip. Ensure that each of the 8 caps fit securely in each of the 8 tubes.
4. Store at 4°C.

Prepare Pierce™ Dilution-Free™ Rapid Gold BCA Working Reagent (WR)

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 40 unknowns and 2 replicates of each sample:

   (8 standards + 40 unknowns) × (2 replicates) × (0.2 mL) = 19.2 mL WR required (Prepare 22 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 Dilution-Free™ Rapid Gold BCA Reagent A with 1 part Dilution-Free™ Rapid Gold BCA Reagent B (50:1, Reagent A:B). For the above example, combine 22 mL of Reagent A with 0.44 mL of Reagent B.

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

   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 provided the WR is used within 1.5 hours.

Perform microplate procedure (sample to WR ratio 1:20)

1. Pipette 10 µL of each standard or unknown sample replicate into a microplate well (working range = 20—10,000 µg/mL) (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Cat. No. 15041).
2. Add 200 µL of the WR to each well, then 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 10 minutes to ensure optimal signal linearity. In the event plate reading must be done after 10 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 minimal impact to results. See Figure 3.

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: 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 absorbance 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 cuvette procedure (sample to WR ratio 1:40)

1. Pipette 25 µL of each standard and unknown sample replicate into appropriately labeled cuvettes. Include a water blank.
2. Add 1 mL of the WR to each cuvette, then mix well (blank = water + WR).
3. Incubate cuvettes 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: If the total number of samples (including standards) are < 15, the absorbance can be measured without having to stop the reaction. If the total number of samples (including standards) is > 15, it is recommended to stop the assay by addition of 250 µL of 1 N HCl after the 5 minute incubation. Once the reaction is stopped, the tubes can 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, a linear curve will provide very accurate results.

Related products

Product	Cat. no.
Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-10 mg/mL	A56979
Pierce™ Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible, 0.125-2 mg/mL	A55863
Pierce™ 96-Well Polystyrene Plates, Corner Notch, 100/pkg	15041
ELISA Reagent Reservoirs	15075
Sealing Tape for 96-Well Plates	15036
Pierce™ BCA Protein Assay Kit with Dilution-Free™ BSA Protein Standards, Multichannel Pipette Compatible	A55865
Micro BCA™ Protein Assay Kit, working range 0.5-20 µg/mL	23235
Qubit™ Protein BR Assay Kit	A50668
Pierce™ Bovine Gamma Globulin Standard Pre-Diluted Set, 7 x 3.5 mL	23213
Varioskan™ LUX multimode microplate reader	VL0000D0

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 (e.g., use 50:2 versus typical 50:1 ratio)
Standards and samples show less color than expected while 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 > 10 mg/mL.	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

Absorbance results

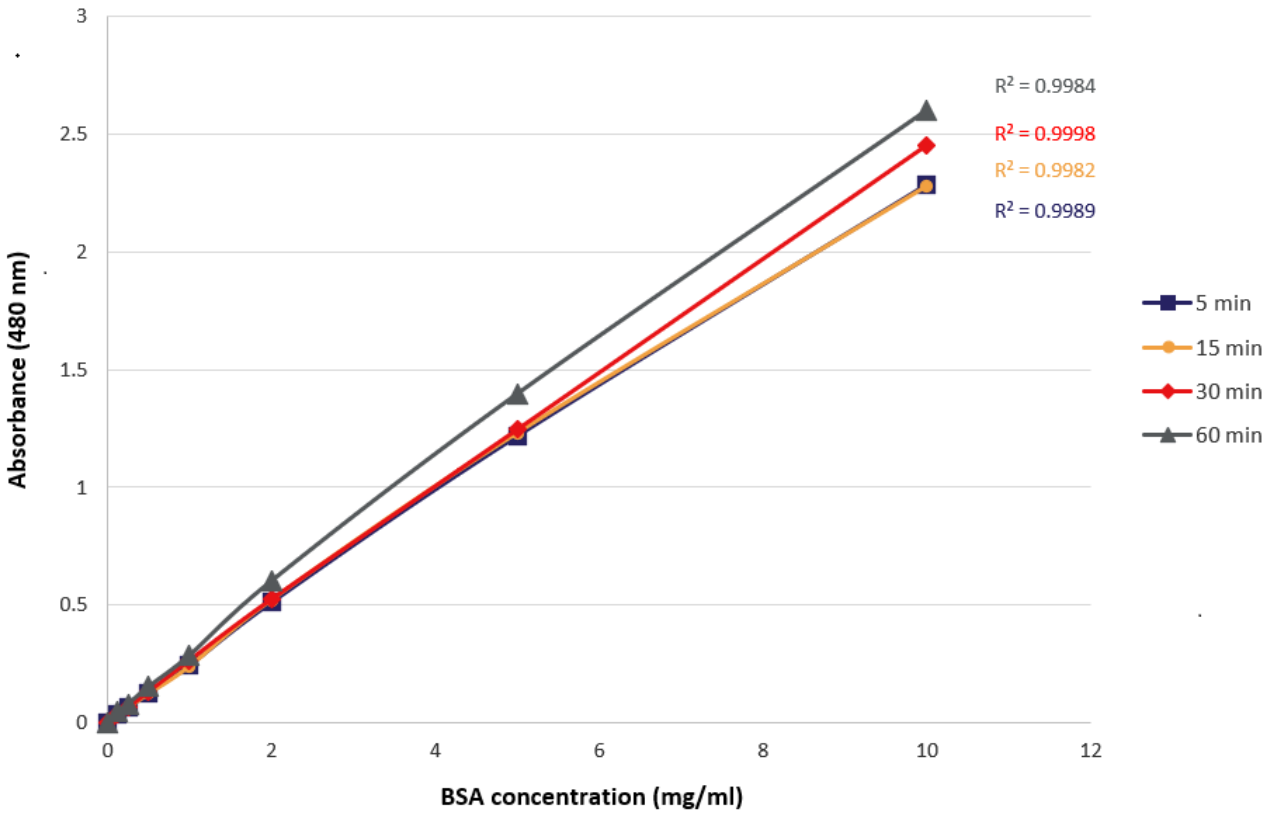

Figure 3 Absorbance at 480 nm read up to 1 hour after incubation. 1 N HCl was used to stop the reaction.

Assay compatibility for various substances

Note: For a more extensive list of substances, download *Tech Tip: Protein Assay Compatibility Table* from our website . This tech tip includes compatible substances for many of our protein assays and enables easy comparisons.

Table 2 Assay compatibility for various substances

Salts/Buffers	Compatible Concentration
ACES, pH 7.8	25 mM
Bicine, pH 8.4	10 mM
Bis-Tris, pH 6.5	10 mM
Borate (50 mM), pH 8.5 (Cat. No. 28384)	undiluted
B-PER™ Reagent (Cat. No. 78248)	undiluted
Calcium chloride in TBS, pH 7.2	10 mM
Na-Carbonate/Na-Bicarbonate (0.2 m0, pH 9.4 (Cat. No. 28382)	undiluted
Cesium bicarbonate	100 mM
CHES, pH 9.0	100 mM
Na-Citrate (0.6 M), MOPS (0.1 M), pH 7.5 (Cat. No. 28386)	1:16 dilution[1]
Na-Citrate (0.6 M), Na-Carbonate (0.1 M), pH 9.0 (Cat. No. 28388)	1:16 dilution[1]
Cobalt chloride in TBS, pH 7.2	0.8 mM
EPPS, pH 8.0	100 mM

Salts/Buffers	Compatible Concentration
Ferric chloride in TBS, pH 7.2	5 mM
Glycine•HCl,pH 2.8	100 mM
Guanidine•HCl	4 M
HEPES, pH 7.5	100 mM
Imidazole,pH 7.0	5 mM
MES, pH 6.1	100 mM
MES (0.1M), NaCl (0.9%), pH 4.7 (Cat. No. 28390)	undiluted
MOPS, pH 7.2	100 mM
Modified Dulbecco's PBS, pH 7.4 (Cat. No. 28374)	undiluted
Nickel chloride in TBS, pH 7.2	10mM
PBS: Phosphate (0.1 M), NaCl (0.15 M), pH 7.2 (Cat. No. 28372)	undiluted
PIPES, pH 6.8	100 mM
RIPA lysis buffer: 50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	undiluted
Sodium acetate, pH 4.8	200 mM
Sodium azide	0.2%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
Sodium citrate, pH 4.8 or pH 6.4	100 mM
Sodium phosphate	100 mM
Tricine,pH 8.0	25 mM
Triethanolamine,pH 7.8	25 mM
Tris	50 mM
TBS:Tris (25 mM), NaCl (0.15 M), pH 7.6 (Cat. No. 28376)	undiluted
Tris (25 mM), Glycine (192 mM), pH 8.0 (Cat. No. 28380)	1:2 dilution[1]

[1] Diluted with ultrapure water

Detergents	Compatible Concentration
Brij™-35	5.0%
Brij™-58	1.0%
CHAPS, CHAPSO	5.0%
Na Deoxycholate (DOC)	5.0%
Octyl β-glucoside	5.0%
SDS	5.0%
Span™-20	1%
Triton-X™-100	5.0%
Triton-X™-114, Triton-X™-305, Triton-X™-405	1.0%
Tween™-20, Tween™-60, Tween™-80	5.0%
Zwittergent™ 3-14	1.0%

Chelating Agents	Compatible Concentration
EDTA	10 mM
Sodium citrate	100 mM

Reducing and Thiol-containing Agents	Compatible Concentration
N-acetylglucosamine in PBS, pH 7.2	10 mM
Glucose	10 mM

Misc. Reagents and Solvents	Compatible Concentration
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF, DMSO	10%
DMSO	10%
Ethanol	10%
Glycerol (fresh)	2%
Hydrochloric acid	100 mM
Leupeptin	10 mg/L

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029413 B.0

Revision	Date	Description
B.0	26 October 2023	Correcting concentrations in Assay Compatibility section.
A.0	3 August 2023	New document for Pierce™ Dilution-Free™ Rapid Gold BCA Protein Assay Kit .