A rapid, sensitive protein assay for the accurate analysis of protein concentrations

Protein quantitation is an integral part of many laboratory workflows and often a necessary step before isolation, separation, and analysis by chromatographic, electrophoretic, or immunochemical techniques. Thermo Scientific™ Pierce™ protein assays provide exceptional accuracy, compatibility, and broad applicability that enable most laboratory protein samples to be quantified (or quantitated) with ease. The latest advance in protein quantitation is the Thermo Scientific™ Pierce™ Rapid Gold BCA Protein Assay. This colorimetric assay provides the high sensitivity and linearity associated with the BCA assay, but in a fraction of the time it takes to perform a standard BCA assay.

An adaptation of the standard BCA assay, the Pierce Rapid Gold BCA Protein Assay has been optimized to perform rapidly at room temperature (RT) to provide ready-to-read results within 5 minutes without the need for incubation at elevated temperatures (Figure 1). Similar to the BCA assay, the Pierce Rapid Gold BCA Protein Assay combines the well-known reduction of Cu^{2+} to Cu^{+} by proteins in an alkaline medium (biuret reaction) with highly sensitive and selective colorimetric detection by a new copper chelator. The amount of Cu^{2+} reduced is proportional to the amount of protein present in the solution. The selective copper chelator forms an orange-gold–colored complex that strongly absorbs light at 480 nm.

Here we present data to demonstrate the performance of the Pierce Rapid Gold BCA Protein Assay and benchmark it against many of the well-known, highly trusted assays for protein quantitation.

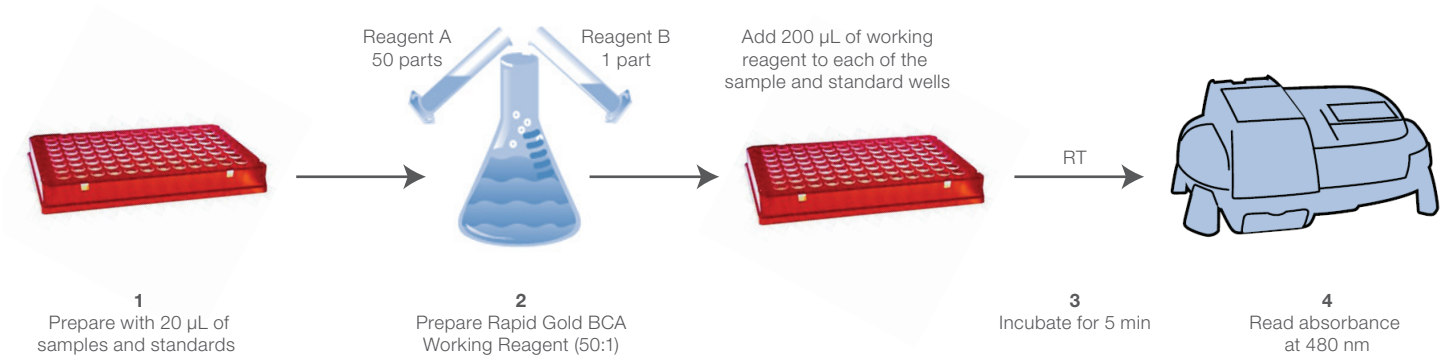

Figure 1. Pierce Rapid Gold BCA Protein Assay protocol.

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Results and discussion

Linearity

In 1985, Smith et al. introduced what is now the Thermo Scientific™ Pierce™ BCA Protein Assay. Since then it has become the most accurate method for colorimetric detection and quantitation of total protein. One of the major advantages of the BCA assay is that it produces a linear response curve. This response curve allows accurate determination of unknown protein concentrations and provides a higher dynamic range than other standard assays. The Pierce Rapid Gold BCA Protein Assay produces a linear response curve ($R^2 > 0.99$) nearly identical to that of the standard BCA, but without the need to incubate samples and in a fraction of the time (Figure 2A). Similar to the standard BCA assay, the Pierce Rapid Gold BCA Protein Assay has a broad dynamic range and can be used to detect protein concentrations from 20 µg/mL to 2,000 µg/mL in tube and microplate formats. Additionally, the Pierce Rapid Gold BCA Protein Assay and the standard BCA assay perform equally, but the new Rapid Gold assay uses less sample (20 µL, compared to 25 µL in the standard BCA assay). When the standard BCA assay is compared to the Pierce Rapid Gold BCA Protein Assay in protein concentration determination, similar concentrations are determined for complex lysates, with a coefficient of variation (CV) between the assays of 5.41% (Figure 3).

Another variety of simple colorimetric assays commonly used for measuring total protein concentration is Coomassie dye–binding assays (e.g., Bradford Protein Assay). These assays are the fastest and simplest to perform, of all protein assays. The assays are performed at room temperature, and no special equipment is required. The Bradford Protein Assay measures the color change of Coomassie brilliant blue G-250 dye when it binds to primarily basic and aromatic amino acid residues. In the acidic environment of the reagent, protein binds to the Coomassie dye, resulting in a spectral shift from the reddish brown form of the dye (absorbance maximum at 465 nm) to the blue form of the dye (absorbance maximum at 595 nm).

Some of the drawbacks to this convenient assay are the nonlinearity of its standard curve, lower dynamic range, and high protein-to-protein variation (Figure 2B). This causes higher error rates and CV values compared to copper-chelating BCA assays. The Pierce Rapid Gold BCA Protein Assay has a greater linear range (20–2,000 µg/mL) than the Bradford Protein Assay (125–1,000 µg/mL) (Figure 2B).

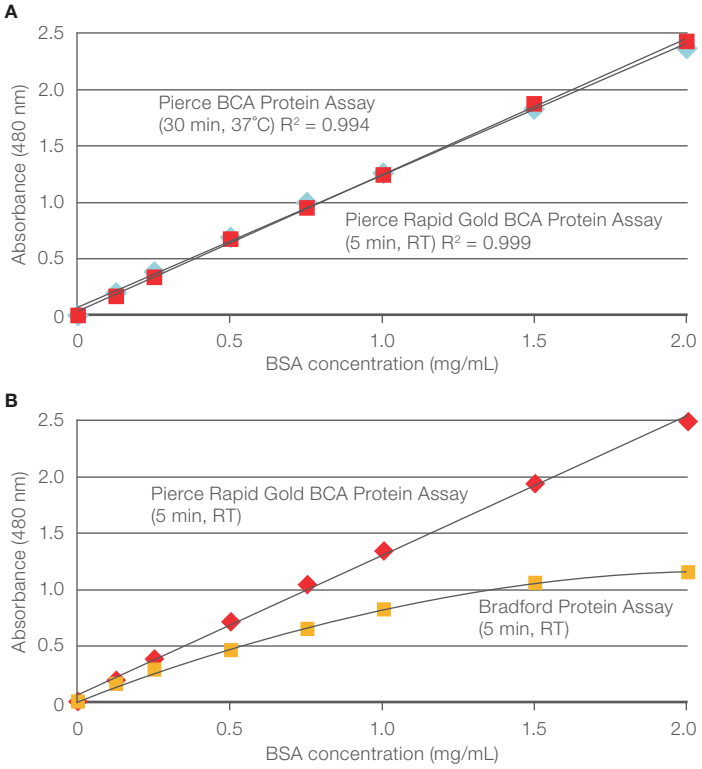

Figure 2. Standard curves for protein quantitation assays. (A) Purified BSA in 0.9% saline (0–2 mg/mL) was used to generate standard curves for the Pierce Rapid Gold BCA Protein Assay and the standard BCA assay. Both assays were conducted according to the manufacturer's protocols, in a microplate format. For the standard BCA assay, 25 µL of BSA sample was added to 200 µL of BCA working reagent and incubated for 30 minutes at 37°C. For the Pierce Rapid Gold BCA Protein Assay, 20 µL of BSA sample was added to 200 µL of Rapid Gold BCA working reagent and incubated at room temperature for 5 minutes. **(B)** The same method described in **(A)** was used to generate standard curves for the Pierce Rapid Gold BCA Protein Assay and Bio-Rad™ Bradford Protein Assay. For the Bradford Protein Assay, 10 µL of BSA sample was added to 200 µL of the Bradford reagent and incubated at room temperature for 5 minutes.

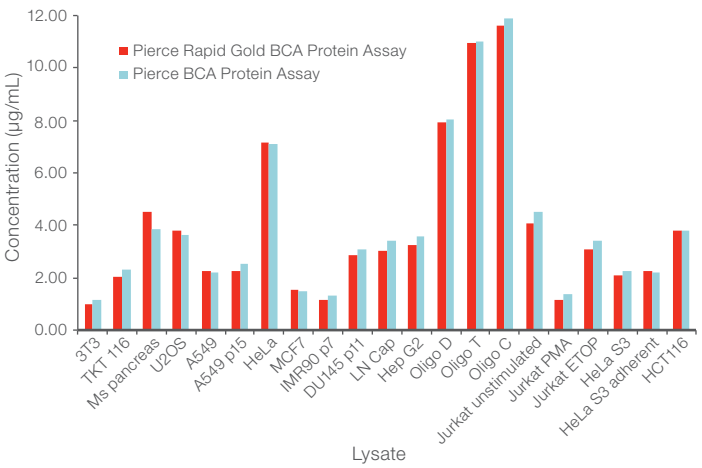

Figure 3. Concentration determination of lysates using the standard Pierce BCA Protein Assay and Pierce Rapid Gold BCA Protein Assay. Both assays were conducted according to the manufacturer's protocols, in a microplate format. For the standard BCA assay, 25 µL of sample was added to 200 µL of BCA working reagent and incubated for 30 minutes at 37°C. For the Pierce Rapid Gold BCA Protein Assay, 20 µL of sample was added to 200 µL of the Pierce Rapid Gold BCA working reagent and incubated at room temperature for 5 minutes.

The Pierce Rapid Gold BCA Protein Assay combines the advantages of Coomassie dye–based assays (fast, easy to perform) with the high quality of BCA assays, which produce higher linearity and more accurate quantitation measurements.

Protein-to-protein variation

One of the highlights of the BCA protein assays is the low protein-to-protein variation. Proteins are diverse in their composition and structure, and with some assays the proteins' differences in amino acid sequence, isoelectric point (pI), secondary structure, and side chains or prosthetic groups result in variation in the colorimetric response. Table 1 shows the relative degree of protein-to-protein variation that can be expected with different protein assay reagents. The differences may be a consideration in selecting a protein assay method, especially if the relative color response ratio of the protein in the samples is unknown. As expected, assays that share the same basic chemistry show similar protein-to-protein variation. For each of the five methods presented here, a group of 14 proteins was assayed using the standard protocol in a single run. The net (blank-corrected) average absorbance for each protein was calculated. The net absorbance for each protein is expressed as a ratio relative to the net absorbance for BSA. If a protein has a ratio of 0.80, it means that the protein produces 80% of the color obtained for an equivalent mass of BSA. The protein-to-protein variation in the amount of color produced with the Pierce Rapid Gold BCA Protein Assay is relatively low, with a CV of 15.6%.

Table 1. Protein-to-protein variation of common protein assays. All of the proteins were tested using the standard tube protocol, using a protein concentration of 1,000 µg/mL. Each number represents the quantitation response relative to that of BSA.

	Pierce Rapid Gold BCA assay	Standard BCA assay	Modified Lowry assay	Coomassie Plus assay	Bradford assay (Bio-Rad)
BSA	1.00	1.00	1.00	1.00	1.00
Aldolase, rabbit muscle	1.22	0.85	0.76	0.74	0.97
α-chymotrypsinogen	1.46	1.14	0.48	0.52	0.41
Cytochrome C, horse heart	1.27	0.83	1.07	1.03	0.48
Gamma globulin, bovine	1.58	1.11	0.56	0.58	0.58
IgG, bovine	1.64	1.21	0.58	0.63	0.65
IgG, human	1.43	1.09	0.63	0.66	0.70
IgG, mouse	1.40	1.18	0.59	0.62	0.60
IgG, rabbit	1.52	1.12	0.37	0.43	0.53
IgG, sheep	1.24	1.17	0.53	0.57	0.53
Insulin, bovine pancreas	1.34	1.08	0.60	0.67	0.14
Myoglobin, horse heart	0.96	0.74	1.09	1.15	0.89
Ovalbumin	1.18	0.93	0.32	0.68	0.27
Transferrin, human	1.16	0.89	0.84	0.90	0.95
Average ratio	1.31	1.02	0.68	0.73	0.60
SD	0.20	0.15	0.26	0.21	0.28
CV (%)	15.6	14.7	38.2	28.8	46.0

This low protein-to-protein variation leads to higher accuracy in determining protein concentration for unknown protein samples. To demonstrate the accuracy of the Pierce Rapid Gold BCA Protein Assay, several different protein mixes were generated with known protein concentrations, using absorbance at 280 nm to confirm concentration. The concentration was determined with the Pierce Rapid Gold BCA Protein Assay and the Bradford Protein Assay at room temperature for 5 minutes (Figure 4). The CV from known concentrations was 20.5% for the Pierce Rapid Gold BCA Protein Assay and 62.2% for the Bradford Protein Assay.

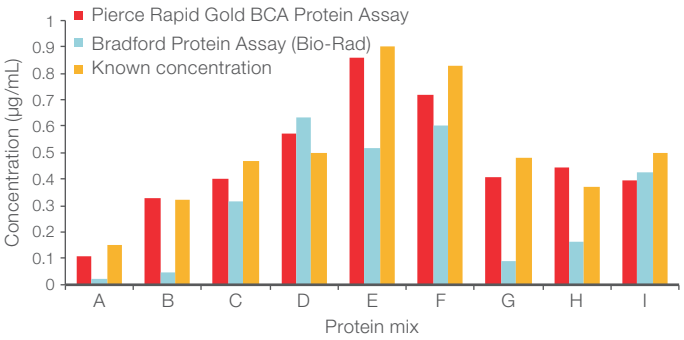

Figure 4. Accuracy of the Pierce Rapid Gold BCA Protein Assay and Bradford Protein Assay with known protein mixes. Both assays were conducted according to the respective manufacturers' protocols, in a microplate format. For the Bradford assay, 10 µL of the BSA sample was added to 200 µL of the Bradford working reagent and incubated at room temperature for 5 minutes. For the Pierce Rapid Gold BCA Protein Assay, 20 µL of sample was added to 200 µL of Rapid Gold BCA working reagent and incubated at room temperature for 5 minutes. Known concentrations were based on manufacturers' indicated concentrations and confirmed by absorbance at 280 nm.

Reagent compatibility

The BCA protein assay has a unique advantage over the Thermo Scientific™ Pierce™ Modified Lowry Protein Assay and any of the Coomassie dye–based assays—it is compatible with samples that contain up to 5% detergents. The Pierce Rapid Gold BCA Protein Assay and the standard BCA assay are similar to each other in their compatibility with surfactants and other commonly used buffers. The Pierce Rapid Gold BCA Protein Assay was carried out using many of the commonly used buffers and contaminants used in typical proteomic laboratories. Table 2 presents a summary of the substances tested and their compatibility.

Table 2. Assay compatibility. To obtain the maximum compatible concentration of substances tested, the assay was performed using the microplate procedure at the midpoint concentration of the BSA standard (1,000 µg/mL). Concentrations listed refer to the actual concentration in the protein sample. Ø denotes compounds that were not compatible at the lowest concentration tested. Dilutions are expressed as undiluted or in the form of a ratio, where "1:2" means 2-fold dilution (e.g. 1 mL of sample in buffer to 1 mL ultrapure water for total of 2 mL). See Table 3 in "Methods" for buffer formulations.

Test compound	Compatibility
2-mercaptoethanol	Ø
ACES (pH 7.8)	25 mM
Acetone	10%
Acetonitrile	10%
Ammonium sulfate	Ø
Aprotinin	10 mg/L
Bicine	20 mM
Borate (pH 8.5)	50 mM
B-PER reagent	Undiluted
Brij-35	5%
Brij-58	1%
Calcium chloride (in TBS, pH 7.2)	10 mM
Carbonate-bicarbonate	1:2 dilution
CHAPS	5%
CHAPSO	5%
CHES	100 mM
Cobalt chloride (in TBS pH 7.2)	0.8 mM
Dithiothreitol (DTT)	Ø
DMF	10%
DMSO	10%
D-PBS	Undiluted
EDTA	10 mM
EPPS (pH 8.0)	100 mM
Ethanol	10%
Ferric chloride (in TBS, pH 7.2)	10 mM
Glucose	10 mM
Glycerol	10%
Glycine-HCl (pH 2.8)	100 mM
Guanidine-HCl	4 M
Hydrochloric acid (HCl)	100 mM
Imidazole (pH 7.0)	12.5 mM
I-PER reagent	Undiluted
Leupeptin	10 mg/L
Mem-PER Plus reagent	Undiluted
MES (pH 6.1)	100 mM
MES-buffered saline (pH 4.7)	Undiluted
Methanol	10%
MOPS (pH 7.2)	100 mM
M-PER reagent	Undiluted
Sodium acetate (pH 4.8)	200 mM
Sodium azide	0.20%
Sodium bicarbonate	100 mM
Sodium chloride	1 M

Table 2. Assay compatibility (continued).

Test compound	Compatibility
Sodium citrate (pH 4.8)	200 mM
Sodium citrate-carbonate (pH 9.0)	1:8
Sodium citrate-MOPS (pH 7.5)	1:8
Sodium deoxycholate (DOC)	5%
Sodium-orthovanadate in PBS (pH 7.2)	1 mM
Sodium phosphate	100 mM
N-acetylglucosamine	10 mM
NE-PER (CER) reagent	1:2 dilution
NE-PER (NER) reagent	Undiluted
NP-40	5%
Octyl β-glucoside	5%
Phosphate-buffered saline (PBS)	Undiluted
PMSF in isopropanol	1 mM
Potassium thiocyanate	3 M
RIPA buffer	Undiluted
SDS	5%
Span 20	0.5%
Sucrose	40%
TLCK	0.1 mg/mL
TPCK	0.1 mg/mL
T-PER reagent	1:2
Tricine (pH 8.0)	25 mM
Triethanolamine (pH 7.8)	25 mM
Tris-buffered saline (TBS)	Undiluted
Tris-glycine (pH 8.0)	1:2
Tris-glycine-SDS (pH 8.3)	Undiluted
Tris-HCl (pH 8.0)	Ø
Tris-HEPES-SDS (pH 7.5)	100 mM
Triton X-100	5%
Triton X-114	1%
Triton X-305	1%
Triton X-405	1%
Tween 20	5%
Tween 60	5%
Tween 80	5%
Urea	3 M
Y-Per Plus reagent	Ø
Y-Per reagent	Ø

Conclusion

The Pierce Rapid Gold BCA Protein Assay is a versatile, accurate, and fast protein quantitation assay that can be completed in 5 minutes at room temperature. Like the "original (standard)" BCA assay, the Pierce Rapid Gold BCA Protein Assay is based on a copper chelator for colorimetric detection and quantitation of total protein. This assay uses the same principle as the standard BCA assay. The Pierce Rapid Gold BCA Protein Assay has less protein-to-protein variability than the Coomassie dye–based assays, with 15% variation among protein standards, whereas the Coomassie dye–based assay demonstrates >40% variation (Table 1). In general, the new Pierce Rapid Gold BCA Protein Assay provides one of the most accurate measurements of protein concentration in biological samples, is detergent compatible, and is simple to perform, with a linear standard curve and a range of 20–2,000 µg/mL.

References

1. Bradford MM (1976) *Anal Biochem* 72:248-254.
2. Smith PK et al. (1985) *Anal Biochem* 150:76-85.

Methods

Pierce Rapid Gold BCA Protein Assay

For each standard or sample, 20 µL was dispensed in replicate into a 96-well microplate. The Pierce Rapid Gold BCA Protein Assay (Cat. No. A53225) working reagent was prepared by mixing 50 parts of reagent A and 1 part of reagent B, and 200 µL of the working reagent was added to each well with a multichannel pipettor and mixed thoroughly on a plate shaker for 30 seconds. The plate was incubated at room temperature for 5 minutes, and absorbance was then detected at 480 nm on a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer. Unknown protein concentrations were determined using a standard curve generated using the Thermo Scientific™ Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set (Cat. No. 23208).

Protein-to-protein variability

The Pierce Rapid Gold BCA Protein Assay (Cat. No. A53225), Pierce BCA Protein Assay (Cat. No. 23225), Thermo Scientific™ Pierce™ Micro BCA™ Protein Assay (Cat. No. 23235), Pierce Modified Lowry Protein Assay (Cat. No. 23240), Thermo Scientific™ Pierce™ Coomassie Plus (Bradford) Assay (Cat. No. 23236), and Bio-Rad Bradford Protein Assay (Cat. No. 50000002) were used according to manufacturers' instructions. The average protein absorbance of 14 commonly used protein standards was determined at a concentration of 1,000 µg/mL. The average absorbance for each protein was compared to the average net absorbance for BSA measured with each kit.

Several different protein mixes were made using commercially available purified proteins with known concentrations, which were verified by measuring absorbance at 280 nm. These commercially available proteins were then mixed in known amounts with other commercially available proteins to generate protein mixtures of known concentrations. The protein mixes were quantitated using the Pierce Rapid Gold BCA Protein Assay (Cat. No. A53225) and the Bio-Rad Bradford Protein Assay (Cat. No. 50000002) at room temperature for 5 minutes according to manufacturers' instructions. The observed protein-mix concentrations were calculated using a standard curved developed with the Pierce Bovine Serum Albumin Standard Pre-Diluted Set (Cat. No. 23208) for each assay.

Reagent compatibility

The Pierce Rapid Gold BCA Protein Assay was performed as described before with samples of 1,000 µg/mL of BSA containing commonly used buffers and contaminants. Assays were performed in triplicate, and absorbance was compared to that of BSA in 0.9% saline. The assay was considered compatible with the tested substance at the indicated concentration if the error in protein concentration estimation caused by the presence of the substance was less than 20%.

Table 3. Buffer formulations used in compatibility testing.

Buffer	Formulation
MES-buffered saline, pH 4.7	0.1 M MES, 150 mM NaCl, pH 4.7
Sodium carbonate-bicarbonate, pH 9.4	0.2 M sodium carbonate-bicarbonate, pH 9.4
Sodium citrate-carbonate, pH 9.0	0.6 M sodium citrate, 0.1 M sodium-carbonate, pH 9.0
Sodium citrate-MOPS, pH 7.5	0.6 M sodium citrate, 0.1 M MOPS, pH 7.5
Phosphate-buffered saline (PBS)	100 mM sodium phosphate, 150 mM NaCl, pH 7.2
RIPA buffer	50 mM Tris, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0
Tris-buffered saline (TBS)	25 mM Tris, 150 mM NaCl, pH 7.6
Tris-glycine, pH 8.0	25 mM Tris, 192 mM glycine, pH 8.0
Tris-glycine-SDS, pH 8.3	25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3
Tris-HEPES-SDS	100 mM Tris, 100 mM HEPES, 3 mM SDS

Find out more at **thermofisher.com/bca-assays**

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