INSTRUCTIONS

Coomassie Plus (Bradford) Assay Kit

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23236

Number	Description
23236	**Coomassie Plus (Bradford) Assay Kit**, sufficient reagents for 630 test tube or 3160 microplate assays

Kit Contents:

Coomassie Plus (Bradford) Assay Reagent, 950mL, containing coomassie G-250 dye, methanol, phosphoric acid and solubilizing agents in water; store at 4°C

Caution: Phosphoric acid is a corrosive liquid.

Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules, containing bovine serum albumin (BSA) at 2.0mg/mL in a solution of 0.9% saline and 0.05% sodium azide; store unopened ampules at room temperature (Available separately as Product No. 23209)

Storage: Upon receipt store each component as indicated. Product shipped at ambient temperature.

Note: Discard any reagent that shows discoloration or evidence of microbial contamination.

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Introduction

The Thermo Scientific™ Coomassie Plus™ Kit is a quick and ready-to-use coomassie-binding, colorimetric method for total protein quantitation. This modification of the well-known Bradford method greatly reduces the tendency of coomassie reagents to give nonlinear response curves by a formulation that substantially improves linearity for a defined range of protein concentration. In addition, the Coomassie Plus Reagent results in significantly less protein-to-protein variation than is observed with other Bradford-type coomassie formulations.

When coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465nm to 595nm with a concomitant color change from brown to blue. Performing the assay in either test tube or microplate format is simple: Combine a small amount of protein sample with the assay reagent, mix well, incubate briefly and measure the absorbance at 595nm. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions, which are assayed alongside the unknown samples.

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Preparation of Standards and Assay Reagent

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably in the same diluent as the sample(s). Each 1mL ampule of Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard.

Table 1. Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube and Microplate Protocols (Working Range = 125–1500µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300µL of Stock	2000µg/mL
B	125µL	375µL of Stock	1500µg/mL
C	325µL	325µL of Stock	1000µg/mL
D	175µL	175µL of vial B dilution	750µg/mL
E	325µL	325µL of vial C dilution	500µg/mL
F	325µL	325µL of vial E dilution	250µg/mL
G	325µL	325µL of vial F dilution	125µg/mL
H	400µL	100µL of vial G dilution	25µg/mL
I	400µL	0	0µg/mL = Blank

Dilution Scheme for Micro Test Tube or Microplate Protocols (Working Range = 1–25µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	3555µL	45µL of Stock	25µg/mL
B	6435µL	65µL of Stock	20µg/mL
C	3970µL	30µL of Stock	15µg/mL
D	3000µL	3000µL of vial B dilution	10µg/mL
E	2500µL	2500µL of vial D dilution	5µg/mL
F	1700µL	1700µL of vial E dilution	2.5µg/mL
G	4000µL	0	0µg/mL = Blank

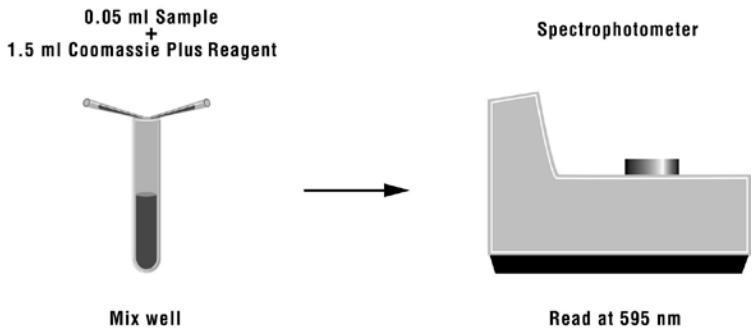
B. Mixing and Equilibrating the Coomassie Plus Reagent

Mix the Coomassie Plus Reagent solution immediately before use by gently inverting the bottle several times (Do not shake the bottle to mix the solution). Remove the amount of reagent needed and equilibrate it to room temperature (RT) before use.

Note: Coomassie Plus Reagent contains additives that retard formation of dye-dye and dye-protein aggregates that tend to form in all coomassie-based protein assay reagents. If left undisturbed, the aggregates will become large enough over time to be visible. For example, when left overnight in a clear glass tube, the reagent forms dye-dye aggregates that are visible as a dark precipitate in the bottom of the tube with nearly colorless liquid above. Dye-dye aggregates can form over several hours in stored reagent while dye-protein-dye aggregates form within seconds. Fortunately, gentle mixing completely disperses the aggregates. Therefore, it is good practice to mix the Coomassie Plus Reagent before dispensing and to mix each tube or plate immediately before measuring absorbances.

Procedure Summary

(Standard Test Tube Protocol):



Test Tube Procedures

A. Standard Test Tube Protocol (Working Range = 125-1500µg/mL)

Note: The linear working range with BSA = 125-1000µg/mL; the linear working range with IgG = 125-1500µg/mL.

1. Pipette 0.05mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5mL of the Coomassie Plus Reagent to each tube and mix well.
3. Optional: For the most consistent results, incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

B. Micro Test Tube Protocol (Working Range = 1-25µg/mL)

1. Pipette 1.0mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.0mL of the Coomassie Plus Reagent to each tube and mix well.
3. Optional: For the most consistent results, incubate samples for 10 minutes at room temperature (RT).
4. With the spectrophotometer set to 595nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedures

A. Standard Microplate Protocol (Working Range = 100-1500µg/mL)

1. Pipette 10µL of each standard or unknown sample into the appropriate microplate wells (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).
2. Add 300µL of the Coomassie Plus Reagent to each well and mix with plate shaker for 30 seconds.
3. Remove plate from shaker. For the most consistent results, incubate plate for 10 minutes at room temperature (RT).
4. Measure the absorbance at or near 595nm with a plate reader.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: When compared to the Standard Test Tube Protocol, 595nm measurements obtained with the Microplate Protocols are lower because the light path is shorter. Consequently, this may increase the minimum detection level of the assay. If higher 595nm measurements are required, use 15µL of standard or sample and 300µL of Coomassie Plus Reagent per well.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

B. Micro Microplate Protocol (Working Range = 1-25µg/mL)

1. Pipette 150µL of each standard or unknown sample into the appropriate microplate wells.
2. Add 150µL of the Coomassie Plus Reagent to each well and mix with plate shaker for 30 seconds.
3. Remove plate from shaker. For the most consistent results, incubate plate for 10 minutes at room temperature (RT).
4. Measure the absorbance at or near 595nm on a plate reader.
5. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard vs. its concentration (µg/ml). Using the standard curve, determine the protein concentration estimate for each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Troubleshooting

Problem	Possible Cause	Solution
Absorbance of Blank is OK, but remaining standards and samples yield lower values than expected	Improper reagent storage	Store reagent refrigerated
	Reagent still cold	Allow reagent to warm to RT
	Absorbance measured at incorrect wavelength	Measure absorbance near 595nm
Absorbances of Blank and standards are OK, but samples yield lower values than expected	Sample protein (peptide) has a low molecular weight (e.g., less than 3000)	Use the Pierce BCA Assay Kit
A precipitate forms in all tubes	Sample contains a surfactant (detergent)	Dialyze or dilute sample or remove interfering substances from sample using Product No. 23215
		Use the Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit (Product No. 23246)
	Samples not mixed well or left to stand for extended time, allowing aggregates to form with the dye	Mix samples immediately before measuring absorbances
All tubes (including Blanks) are dark blue	Strong alkaline buffer raises pH of formulation, or sample volume too large, thereby raising reagent pH	Dialyze or dilute sample
		Remove interfering substances from sample using Product No. 23215
Need to read absorbances at a different wavelength	Spectrophotometer or plate reader does not have 595nm filter	Color may be read at any wavelength between 575nm and 615nm, although the slope of standard curve and overall assay sensitivity will be reduced

A. Interfering substances

Certain substances are known to interfere with coomassie-based protein assays including most ionic and nonionic detergents, which reduce color development and can cause precipitation of the assay reagent. Other substances interfere to a lesser extent. These have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the Standard Test Tube Protocol are listed in Table 2 (see last page). Substances were compatible in the Standard Test Tube Protocol if the error in protein concentration estimation (of BSA at 1000µg/mL) caused by the presence of the substance in the sample was less than or equal to 10%. The Blank-corrected 595nm absorbance measurements (for the 1000µg/mL BSA standard + substance) were compared to the net 595nm absorbances of the 1000µg/mL BSA standard prepared in 0.9% saline.

B. Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the Coomassie Plus Assay may be overcome by one of several methods.

- Remove the interfering substance by dialysis or desalting.
- Dilute the sample until the substance no longer interferes.
- Precipitate proteins with acetone or trichloroacetic acid (TCA). Upon precipitation the liquid containing the substance that interfered is discarded and the protein pellet is solubilized in a small amount of ultrapure water or directly in the Coomassie Plus Reagent. Alternatively, use Product No. 23215 (see Related Thermo Scientific Products).

  Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

  Note: The Pierce Detergent Compatible Bradford Assay Kit (Product No. 23246) is an alternative related product compatible with a wide range of detergents.

Related Thermo Scientific Products

15041	**Pierce 96-Well Plates – Corner Notch,** 100/pkg
15075	**Reagent Reservoirs,** 200/pkg
15036	**Sealing Tape for 96-Well Plates,** 100/pkg
23208	**Pre-Diluted Bovine Serum Albumin (BSA) Set**, 7 × 3.5mL
23209	**Bovine Serum Albumin Standard Ampules,** 2mg/mL, 10 ×1mL
23212	**Bovine Gamma Globulin Standard Ampules**, 2mg/mL, 10 × 1mL
23213	**Pre-Diluted Bovine Gamma Globulin Fraction II (BGG) Set**, 7 × 3.5mL
23246	**Pierce Detergent Compatible Bradford Assay Kit**
23227	**Pierce BCA Protein Assay Kit**
23235	**Micro BCA™ Protein Assay Kit**
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**

Additional Information

A. Please visit the web site for additional information on this product including:

- Tech Tip #9: Quantitate immobilized protein
- Application notes and more complete reference list

B. 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, isoelectric point, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. Most protein assay methods utilize BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined. Thermo Scientific™ Bovine Serum Albumin Standard (BSA) (Product No. 23209) provides a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Coomassie Plus Assay (Figure 1). For greatest accuracy, the standard curve should be prepared from a pure sample of the target protein to be measured.

Table 3 shows typical protein-to-protein variation in color response. All proteins were tested at 1000µg/mL using the Standard Test Tube Protocol. 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. The protein-to-protein variation observed with the Coomassie Plus Reagent is significantly less than that seen with other Bradford-type coomassie formulations.

C. Measuring Absorbances at Wavelengths other than 595nm

If a photometer or plate reader is not available with a 595nm filter, the blue color may be measured at any wavelength between 570nm and 610nm. The maximum sensitivity of the assay occurs when the absorbance of the dye-protein complex is measured at 595nm. Measuring the absorbance at any wavelength other than 595nm will result in a lower slope for the standard curve and may increase the minimum detection level for the protocol.

D. Effect of Temperature on 595nm Absorbance

Absorbance measurements at 595nm obtained with the Coomassie Plus Reagent are dependent on the temperature of the reagent to some extent. As the reagent temperature increases to RT, the 595nm measurements will increase. Therefore, it is important that the Coomassie Plus Reagent remain at a constant temperature (i.e., RT) during the assay.

E. Cleaning and Re-using Glassware

Care must be exercised when cleaning glassware that will be used again for protein assays. Thorough cleaning often requires the use of a detergent such as Thermo Scientific PCC-54 Detergent (Product No. 72288), which must be completely removed in the final rinse. Coomassie dye will stain glass or quartz cuvettes. Disposable polystyrene cuvettes are a convenient alternative.

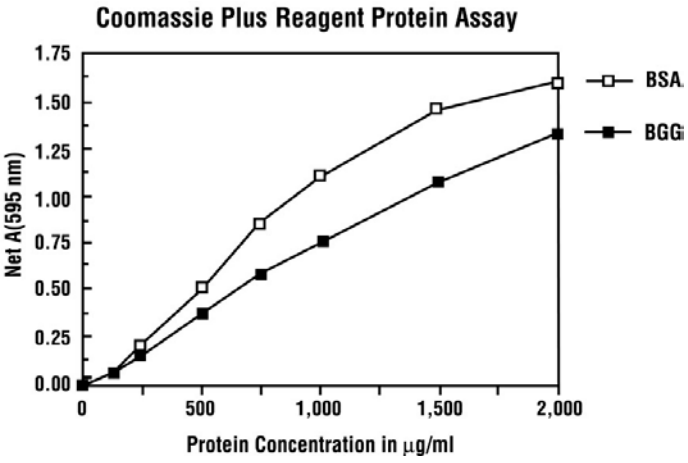


Figure 1. Typical color response curves for BSA and BGG using the Standard Test Tube Protocol.

Table 3. Protein-to-Protein Variation. Absorbance ratios (595nm) for proteins relative to BSA using the Standard Test Tube Protocol in the Coomassie (Bradford) Assay.

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)

Protein Tested	Ratio
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.74
α-Chymotrypsinogen, bovine	0.52
Cytochrome C, horse heart	1.03
Gamma globulin, bovine	0.58
IgG, bovine	0.63
IgG, human	0.66
IgG, mouse	0.62
IgG, rabbit	0.43
IgG, sheep	0.57
Insulin, bovine pancreas	0.67
Myoglobin, horse heart	1.15
Ovalbumin	0.68
Transferrin, human	0.90
Average ratio	**0.73**
Standard Deviation	**0.21**
Coefficient of Variation	**28.8%**

General References

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248-54.

Compton, S.J. and Jones, C.J. (1985) Mechanism of dye response and interference in the Bradford protein assay. *Anal Biochem* **151**:369-74.

Davies, E.M.(1988). Protein assays: A review of common techniques. *Amer Biotech Lab* **July** 28-37.

Sedmak, J.J. and Grossberg, S.E. (1977) A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue G-250. *Anal Biochem* **79**:544-52.

Sherwood, J.K., *et al.* (1996) Controlled release of antibodies for long-term topical passive immunoprecipitation of female mice against genital herpes. *Nature Biotech* **14**:468-71.

Sorensen, K. (1994) Coomassie protein assay used for quantitative determination of sodium cyanoborohydride ($NaCNBH_3$). *Anal Biochem.***218**:231-33.

Tal, M., Silberstein, A. and Nusser, E. (1980) Why does Coomassie brilliant blue R interact differently with different proteins? *J Biol Chem* **260**:9976-80.

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Kagan, A., *et al.* (2000) The dominant negative LQT2 mutation A561V reduces wild-type HERG expression. *J Biol Chem* **275**:11241-8.

Glover, B.P. and McHenry, C.S. (2001) The DNA polymerase III holoenzyme: an asymmetic dimerc replicative complex with leading and lagging strand polymerases. *Cell* **105**:925-34.

Table 2. Compatible substance concentrations in the Coomassie Plus Protein Assay.

Substance	Compatible Concentration
Detergents	
Brij™-35	0.062%
Brij-56 (Brij-58)	0.031%(0.016%)
CHAPS, CHAPSO	5.0%
Deoxycholic acid	0.4%
Lubrol™ PX	0.031%
Octyl β-glucoside	0.5%
Nonidet P-40 (NP-40)	0.5%
Octyl β-thioglucopyranoside	3.0%
SDS	0.016%
Span™ 20	0.5%
Triton™ X-100, X-114	0.062%
Triton X-305, X-405	0.125%(0.025%)
Tween™-20	0.031%
Tween-60	0.025%
Tween-80	0.016%
Zwittergent™ 3-14	0.025%
Chelating agents	
EDTA	100mM
EGTA	2mM
Sodium citrate	200mM
Reducing & Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	100mM
Ascorbic acid	50mM
Cysteine	10mM
Dithioerythritol (DTE)	1mM
Dithiothreitol (DTT)	5mM
Glucose	1.0M
Melibiose	100mM
2-Mercaptoethanol	1.0M
Potassium thiocyanate	3.0M
Thimerosal	0.01%
Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%
Aprotinin	10 mg/L
DMF, DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrochloric Acid	100mM
Leupeptin	10mg/L
Methanol	10%
Phenol Red	0.5mg/mL
PMSF	1mM
Sodium Hydroxide	100mM
Sucrose	10%
TLCK	0.1mg/L
TPCK	0.1mg/L
Urea	3.0M
o-Vanadate (sodium salt), in PBS, pH 7.2	1mM

Substance	Compatible Concentration
Salts/Buffers	
ACES, pH 7.8	100mM
Ammonium sulfate	1.0M
Asparagine	10mM
Bicine, pH 8.4	100mM
Bis-Tris, pH 6.5	100mM
Borate (50mM), pH 8.5 (# 28384)	undiluted
B-PER™ Reagent (#78248)	1/2 dilution*
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4 (#28382)	undiluted
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0 (#28388)	undiluted
Cobalt chloride in TBS, pH 7.2	10mM
EPPS, pH 8.0	100mM
Ferric chloride in TBS, pH 7.2	10mM
Glycine	100mM
Guanidine•HCl	3.5M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	200mM
MES, pH 6.1	100mM
MES (0.1M), NaCl (0.9%), pH 4.7 (#28390)	undiluted
MOPS, pH 7.2	100mM
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted
Nickel chloride in TBS, pH 7.2	10mM
PBS; Phosphate (0.1M), NaCl (0.15M), pH 7.2 (#28372)	undiluted
PIPES, pH 6.8	100mM
RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	1/40 dilution*
Sodium acetate, pH 4.8	180mM
Sodium azide	0.5%
Sodium bicarbonate	100mM
Sodium chloride	5.0M
Sodium citrate, pH 4.8 or pH 6.4	200mM
Sodium phosphate	100mM
Tricine, pH 8.0	100mM
Triethanolamine, pH 7.8	100 mM
Tris	2.0M
TBS; Tris (25mM), NaCl (0.15M), pH 7.6 (#28376)	undiluted
Tris (25mM), Glycine (192mM), pH 8.0 (#28380)	undiluted
Tris (25mM), Glycine (192mM), SDS (0.1%), pH 8.3 (#28378)	1/4 dilution*
Zinc chloride in TBS, pH 7.2	10mM

*Diluted with ultrapure water.