Product information

The Celltechgen™ Coomassie (Bradford) Assay Kit

Cat. No. CTG-PA0017-A	The Celltechgen™ Coomassie (Bradford) Assay Kit,500ml	ISSUE DATE July 9, 2019
Cat. No. CTG-PA0017-B	The Celltechgen™ Coomassie (Bradford) Assay Kit,1000ml	ISSUE DATE July 9, 2019

Kit Contents:

Coomassie (Bradford) Protein 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 a concentration of 2mg/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.

Introduction

The Thermo Scientific™ Coomassie (Bradford) Protein Assay Kit is a quick and ready-to-use modification of the well-known Bradford coomassie-binding, colorimetric method for total protein quantitation. 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. Because the color response with coomassie is non-linear with increasing protein concentration, a standard curve must be completed with each assay.

Preparation of Standards and Working Reagent (required for both assay procedures)

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 Protocol and Microplate Procedure (Working Range = 20-2,000µg/mL)

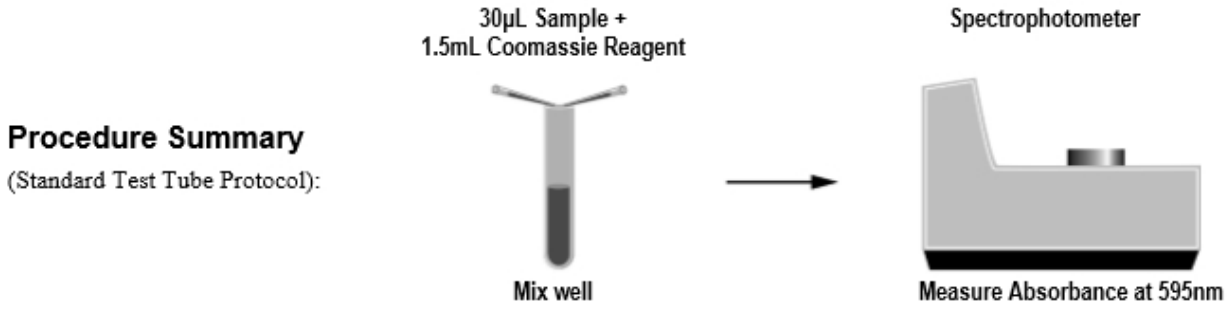
Vial	Volume of Diluent(uL)	Volume and Source of BSA(ul)	Fina BSA Concentration(ug/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vialG dilution	25
I	400	0	0=Blank

Dilution Scheme for Micro Test Tube or Microplate Protocols (Working Range = 1–25µg/mL)			
Vial	**Volume of Diluent(uL)**	**Volume and Source of BSA(ul)**	**Fina BSA Concentration(ug/mL)**
A	2370	30 of Stock	25
B	4950	50 of vial A dilution	20
C	3970	30 of vialB dilution	15
D	2500	2500of vialC dilution	10
E	2000	2000of vialD dilution	5
F	1500	1500of vialE dilution	2.5
G	5000	0	0=Blank

B. Equilibrating and Mixing of the Coomassie Reagent

Mix the Coomassie 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: Dye-dye and dye-protein aggregates tend to form in all coomassie-based 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 more quickly. Fortunately, gentle mixing completely disperses the dye-dye aggregates. Therefore, it is good practice to mix the Coomassie Reagent before pipetting and to mix each tube or plate immediately before measuring absorbances.



Test-tube Procedure

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

1. Pipette 0.03mL (30µL) of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5mL of the Coomassie 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 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 Procedure

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

1. Pipette 5µL of each standard or unknown sample into the appropriate microplate wells (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).

2. Add 250µL of the Coomassie 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 used is shorter. Consequently, this may increase the minimum detection level of the assay. If higher 595nm measurements are required, use 7-10µL of standard or sample and 250µL of Coomassie 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 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).

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 BCA or Lowry Protein Assay
A precipitate forms in all tubes	Sample contains a surfactant(detergent)	Dialyze or dilute sample
	Samples not mixed well or left to stand for extended time, allowing aggregates to form with the dye	Mix samples immediately prior to 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
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 575nmand 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 the1000µg/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 Assay may be overcome by 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 Reagent.

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

Additional Information

A. 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. Albumin Standard Ampules (BSA) provide a consistent standard for protein estimations. Nevertheless, individual proteins, including BSA and IgG, differ slightly in their color responses in the Coomassie Assay. 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 a concentration of 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 Reagent is significantly less than that seen with other Bradford-type coomassie dye

formulations.

B. 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.

C. Effect of Temperature on 595nm Absorbance

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

D. 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, which must be completely removed in the final rinse. The coomassie dye will stain glass or quartz cuvettes. Disposable polystyrene cuvettes are a convenient alternative.

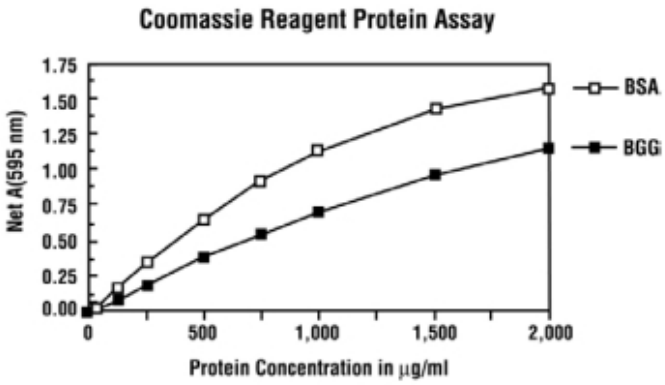


Figure 1. Typical color response curves for BSA and BGG using the Standard Test Tube Protocol of the Coomassie Assay.

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

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)	
Protein Tested	**Ratio**
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.76
α-Chymotrypsinogen, bovine	0.48
Cytochrome C, horse heart	1.07
Gamma globulin, bovine	0.56
IgG, bovine	0.58
IgG, human	0.63
IgG, mouse	0.59
IgG, rabbit	0.37
IgG, sheep	0.53
Insulin, bovine pancreas	0.60
Myoglobin, horse heart	1.19
Ovalbumin	0.32
Transferrin, human	0.84
	0.68
Standard Deviation	**0.26**
Coefficient of Variation	**38.2%**