INSTRUCTIONS

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

# Coomassie (Bradford) Protein Assay Kit

23200

0129.4

| Number | Description |
|---|---|
| **23200** | **Coomassie (Bradford) Protein Assay Kit**, sufficient reagents for 630 test tube assays or 3,800 microplate assays |

**Kit Contents:**

**Coomassie (Bradford) Protein Assay Reagent**, 950 ml, 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, 2 mg/ml**, 10 x 1 ml ampules, containing bovine serum albumin (BSA) at a concentration of 2.0 mg/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

Coomassie (Bradford) 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 465 nm to 595 nm 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 595 nm. 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 Kit is non-linear with increasing protein concentration, a standard curve must be run with each assay.

## 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 1 ml 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 = 100–1,500 µg/ml) | | | |
|---|---|---|---|
| **Vial** | **Volume of Diluent** | **Volume and Source of BSA** | **Final BSA Concentration** |
| A | 0 | 300 µl of Stock | 2,000 µg/ml |
| B | 125 µl | 375 µl of Stock | 1,500 µg/ml |
| C | 325 µl | 325 µl of Stock | 1,000 µg/ml |
| D | 175 µl | 175 µl of vial B dilution | 750 µg/ml |
| E | 325 µl | 325 µl of vial C dilution | 500 µg/ml |
| F | 325 µl | 325 µl of vial E dilution | 250 µg/ml |
| G | 325 µl | 325 µl of vial F dilution | 125 µg/ml |
| H | 400 µl | 100 µl of vial G dilution | 25 µg/ml |
| I | 400 µl | 0 | 0 µg/ml = Blank |
| Dilution Scheme for Micro Test Tube or Microplate Protocols (Working Range = 1–25 µg/ml) | | | |
| **Vial** | **Volume of Diluent** | **Volume and Source of BSA** | **Final BSA Concentration** |
| A | 2,370 µl | 30 µl of Stock | 25 µg/ml |
| B | 4,950 µl | 50 µl of Stock | 20 µg/ml |
| C | 3,970 µl | 30 µl of Stock | 15 µg/ml |
| D | 2,500 µl | 2,500 µl of vial B dilution | 10 µg/ml |
| E | 2,000 µl | 2,000 µl of vial D dilution | 5 µg/ml |
| F | 1,500 µl | 1,500 µl of vial E dilution | 2.5 µg/ml |
| G | 5,000 µl | 0 | 0 µg/ml = Blank |

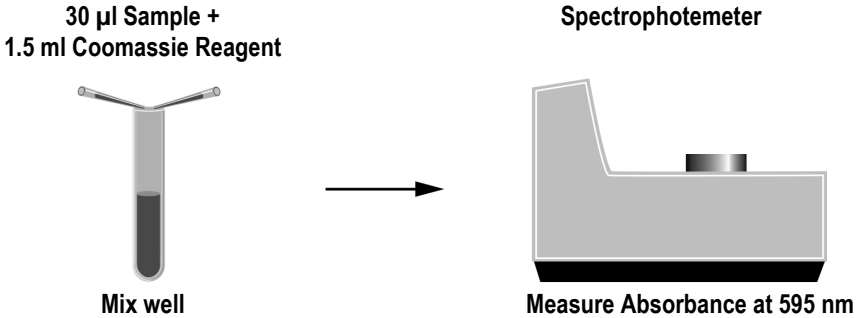
### B. Equilibrating and Mixing of the Coomassie Reagent

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.

## Procedure Summary

(Standard Test Tube Protocol):



## Test Tube Procedures

**A. Standard Test Tube Protocol (Working Range = 100-1,500 µg/ml)**

1. Pipette 0.03 ml (30 µl) of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5 ml 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 595 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Subtract the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595 nm 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 = 1-25 µg/ml)**

1. Pipette 1.0 ml of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.0 ml 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 595 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Subtract the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595 nm 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 = 100-1,500 µg/ml)**

1. Pipette 5 µl of each standard or unknown sample into the appropriate microplate wells.
2. Add 250 µl 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 595 nm with a plate reader.
5. Subtract the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 595 nm 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, 595 nm 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 595 nm measurements are required, use 7-10 µl of standard or sample and 250 µl 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 = 1-25 µg/ml)**

1. Pipette 150 µl of each standard or unknown sample into the appropriate microplate wells.
2. Add 150 µl 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 595 nm on a plate reader.

5. Subtract the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank corrected 595 nm measurement for each BSA standard vs. its concentration in 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 595 nm |
| 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 3,000) | Use the BCA or Lowry Protein Assay |
| A precipitate forms in all tubes | Sample contains a surfactant (detergent) | Dialyze or dilute sample<br>Remove interfering substances from sample using Product No. 23215 |
| | 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<br>Remove interfering substances from sample using Product No. 23215 |
| Need to read absorbances at a different wavelength | Spectrophotometer or plate reader does not have 595 nm filter | Color may be read at any wavelength between 575 nm and 615 nm, 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 1,000 µg/ml) caused by the presence of the substance in the sample was less than or equal to 10%. The Blank-corrected 595 nm absorbance measurements (for the 1,000 µg/ml BSA standard + substance) were compared to the net 595 nm absorbances of the 1,000 µg/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. Alternatively, use Pierce Product No. 23215 (see Related Pierce Products).

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

## Related Pierce Products

| | |
|---|---|
| **23208** | **Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set**, 7 x 3.5 ml of dilutions in the range of 125-2,000 µg/ml |
| **23212** | **Bovine Gamma Globulin Standard Ampules**, 2 mg/ml, 10 x 1 ml |
| **23213** | **Pre-Diluted Protein Assay Standards: Bovine Gamma Globulin Fraction II (BGG) Set**, 7 x 3.5 ml of dilutions in the range of 125-2,000 µg/ml |
| **23227** | **BCA Protein Assay Kit**, working range of 20-2,000 µg/ml |
| **23235** | **Micro BCA™ Protein Assay Kit**, working range 0.5-20 µg/ml |
| **23215** | **Compat-Able™ Protein Assay Preparation Reagent Set**, sufficient reagents to pre-treat 500 samples to remove interfering substances prior to total protein quantitation |

## Additional Information

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

- Frequently Asked Questions
- Tech Tip protocol: Quantitate Protein Immobilized on a Solid Support
- 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. Pierce 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 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 a concentration of 1,000 µg/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.

**C. Measuring Absorbances at Wavelengths other than 595 nm**

If a photometer or plate reader is not available with a 595 nm filter, the blue color may be measured at any wavelength between 570 nm and 610 nm. The maximum sensitivity of the assay occurs when the absorbance of the dye-protein complex is measured at 595 nm. Measuring the absorbance at any wavelength other than 595 nm 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 595 nm Absorbance**

Absorbance measurements at 595 nm 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 595 nm measurements will increase. Therefore, it is important that the Coomassie 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 Pierce Product No. 72288), 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.

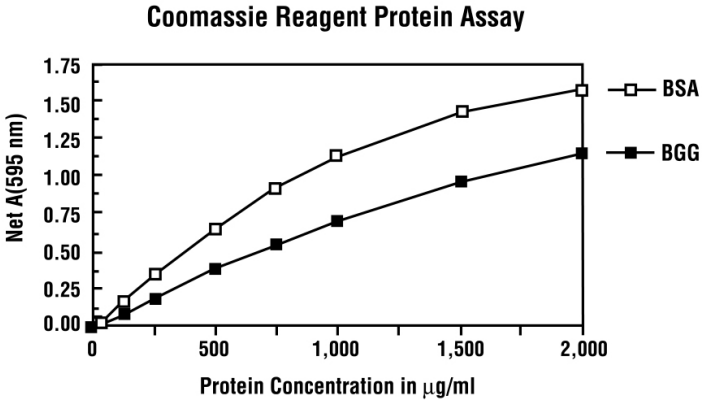



**Figure 1:** Typical color response curves for BSA and BGG using the Standard Test Tube Protocol of the Coomassie Assay.

**Table 3.** Protein-to-Protein Variation. Absorbance ratios (595 nm) 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 |
| **Average ratio** | **0.68** |
| **Standard Deviation** | **0.26** |
| **Coefficient of Variation** | **38.2%** |

## 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.

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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.

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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Ikemoto, A., Bole, D.G. and Ueda, T. (2003). Glycolysis and glutamate accumulation into synaptic vesicles. *J. Biol. Chem*. **278**:5929-40.

Messenger, M.M., *et al.* (2002). Interactions between protein kinase CK2 and Pin1. *J. Biol. Chem*. **277**:23054-64.

Tlapak-Simmons, V.L., *et al.* (1999). Kinetic characterization of the recombinant hyaluronan synthases from Streptococcus pyogenes and Streptococcus equisimilis. *J. Biol. Chem*. **274**:4246-53.

**Table 2.** Compatible Substance Concentrations in the Coomassie Assay (see text for details).

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

| Substance | Compatible Concentration |
|---|---|
| **Detergents** | |
| Brij®-35 | 0.125% |
| Brij®-56, Brij®-58 | 0.031% |
| CHAPS, CHAPSO | 5.0% |
| Deoxycholic acid | 0.05% |
| Lubrol® PX | 0.125% |
| Octyl β-glucoside | 0.5% |
| Nonidet P-40 (NP-40) | 0.5% |
| Octyl β-thioglucopyranoside | 3.0% |
| SDS | 0.125% |
| Span® 20 | 0.5% |
| Triton® X-100, X-114 | 0.125% |
| Triton® X-305, X-405 | 0.5% |
| Tween®-20 | 0.062% |
| Tween®-60 | 0.1% |
| Tween®-80 | 0.062% |
| Zwittergent® 3-14 | 0.025% |
| **Chelating agents** | |
| EDTA | 100 mM |
| EGTA | 2 mM |
| Sodium citrate | 200 mM |
| **Reducing & Thiol-Containing Agents** | |
| *N*-acetylglucosamine in PBS, pH 7.2 | 100 mM |
| Ascorbic acid | 50 mM |
| Cysteine | 10 mM |
| Dithioerythritol (DTE) | 1 mM |
| Dithiothreitol (DTT) | 5 mM |
| Glucose | 1.0 mM |
| Melibiose | 100 mM |
| 2-Mercaptoethanol | 1.0 M |
| Potassium thiocyanate | 3.0 M |
| Thimerosal | 0.01% |
| **Misc. Reagents & Solvents** | |
| Acetone | 10% |
| Acetonitrile | 10% |
| Aprotinin | 10 mg/L |
| DMF, DMSO | 10% |
| Ethanol | 10% |
| Glycerol (Fresh) | 10% |
| Hydrochloric Acid | 100 mM |
| Leupeptin | 10 mg/L |
| Methanol | 10% |
| Phenol Red | 0.5 mg/ml |
| PMSF | 1 mM |
| Sodium Hydroxide | 100 mM |
| Sucrose | 10% |
| TLCK | 0.1 mg/L |
| TPCK | 0.1 mg/L |
| Urea | 3.0 M |
| *o*-Vanadate (sodium salt), in PBS, pH 7.2 | 1 mM |

*Diluted with ultrapure water.