Quick Start™ Bradford Protein Assay

Instruction Manual

For technical service call your local Bio-Rad office, or in the US, 1-800-4BIORAD (1-800-424-6723)

BIO-RAD

Table of Contents

Section 1
Introduction

The Quick Start Bradford protein assay is a simple and accurate procedure for determining the concentration of protein in solution. It provides ready-to-use convenience by supplying the dye reagent at 1x concentration and two protein assay standards at seven prediluted concentrations. The prediluted standards are conveniently packaged in 2 ml screwcap vials, eliminating wasteful and sharp ampoules, and ensuring protein stability over the shelf life of the product.

1.1 Principle

The Bradford assay is a protein determination method that involves the binding of Coomassie

Brilliant Blue G-250 dye to proteins (Bradford 1976). The dye exists in three forms: cationic (red), neutral (green), and anionic (blue) (Compton and Jones 1985). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (A_{max} = 470 nm). However, when the dye binds to protein, it is converted to a stable unprotonated blue form (A_{max} = 595 nm) (Reisner et al. 1975, Fazekes de St. Groth et al. 1963, Sedmack and Grossberg 1977). It is this blue protein-dye form that is detected at 595 nm in the assay using a spectrophotometer or microplate reader.

	H^+		H^+	
Cation	$\leftrightarrow$	Neutral form	$\leftrightarrow$	Anion
470 nm (red)		650 nm (green)		595 nm (blue)

Work with synthetic polyamino acids indicates that Coomassie Brilliant Blue G-250 dye binds primarily to basic (especially arginine) and aromatic amino acid residues (Compton and Jones 1985). Spector (1978) found that the extinction coefficient of a dye-albumin complex solution was constant over a 10-fold concentration range. Thus, Beer's law may be applied for accurate quantitation of protein by selecting an appropriate ratio of dye volume to sample concentration.

Certain chemical-protein and chemical-dye interactions interfere with the assay. Interference from non-protein compounds is due to their ability to shift the equilibrium levels of the dye among the three colored species. Known sources of interference,

such as some detergents, flavonoids, and basic protein buffers, stabilize the green neutral dye species by direct binding or by shifting the pH (Compton and Jones 1985, Fanger 1987). Nevertheless, many chemical reagents do not directly affect the development of dye color when used in the standard protocol and the more common reagents are listed in Table 1. The microassay is compatible with lower concentrations of reagents $^{1}/_{25}$ than listed in Table 1 due to the larger sample volume-to-dye ratio. Since every protein-chemical reagent combination has not been assayed, it is possible that some of the listed reagents interfere in combination with certain proteins. However, with respect to proteins such as bovine serum albumin (BSA) and bovine

gamma-globulin, the listed reagents show little or no interference.

1.2 Selecting a Protein Standard

In any protein assay, the ideal protein to use as a standard is a purified preparation of the protein being assayed. In the absence of such an absolute reference protein, another protein must be selected as a relative standard. The best relative standard to use is one that gives a color yield similar to that of the protein being assayed. Selecting such a protein standard is generally done empirically. Alternatively, if only relative protein values are desired, any purified protein may be selected as a standard. The two most common protein standards used for protein assays are BSA and gamma-globulin.

With the Quick Start Bradford protein assay, dye color development is significantly greater with BSA than with most other proteins, including gamma-globulin. Therefore, the BSA standard would be an appropriate standard if the sample contains primarily albumin, or if the protein being assayed gives similar response to the dye. For a color response that is typical of many proteins, the gamma-globulin standard is appropriate.

Table 1. Reagents compatible with the Quick Start Bradford protein assay when using the standard procedure.*

Acetone, 10%
Acetonitrile, 10%
Ammonium sulfate, 1 M
Ampholytes, 3–10, 0.5%
ASB-14, 0.025%
Ascorbic acid, 50 mM
Bis-Tris, pH 6.5, 0.2 M
β-mercaptoethanol, 1 M
Calcium chloride, 40 mM
CHAPS, 10%
CHAPSO, 10%
Deoxycholic acid, 0.2%
DMSO, 5%
Dithioerythritol (DTE), 10 mM
Dithiothreitol (DTT), 10 mM
Eagle's MEM
Earle's salt solution
EDTA, 0.2 M
EGTA, 0.2 M
Ethanol, 10%
Glucose, 20%
Glycerol, 5%
Glycine, 0.1 M
Guanidine-HCl, 2 M
Hank's salt solution
HCl, 0.1 M
HEPES, 0.1 M
Imidazole, 0.2 M
Magnesium chloride, 1 M
MES, 0.1 M
Methanol, 10%
Modified Dulbecco's PBS
MOPS, 0.1 M
NAD, 2 mM
Nonidet P-40, 0.25%
Octyl β-glucoside, 0.5%
Octyl β-thioglucopyranoside, 1%
PBS

Phenol Red, 0.5 mg/ml
PIPES, 0.2 M
PMSF, 2 mM
Potassium chloride, 2 M
Potassium phosphate, 0.5 M
SB 3–10, 0.1%
SDS, 0.025%
Sodium acetate, pH 4.8, 0.2 M
Sodium azide, 0.5%
Sodium bicarbonate, 0.2 M
Sodium carbonate, 0.1 M
Sodium chloride, 2.5 M
Sodium citrate, pH 4.8 or 6.4, 0.2 M
Sodium hydroxide, 0.1 M
Sodium phosphate, 0.5 M
Sucrose, 10%
TBP, 5 mM
TBS (25 mM Tris, 0.15 M NaCl, pH 7.6), 0.5x
TCEP, 20 mM
Thio-urea, 1 M
Tricine, pH 8, 50 mM
Triethanolamine, pH 7.8, 50 mM
Tris, 1 M
Tris-glycine (25 mM Tris, 192 mM glycine)
Tris-glycine-SDS, (25 mM Tris, 192 mM glycine, 0.1% SDS), 0.5x
Triton X-100, 0.05%
Tween 20, 0.01%
Urea, 4 M

*The concentration limits for compatibility with the microassay are $^{1}/_{25}$ of the values in Table 1.

1.3 Product Description

All kit components have a 1 year shelf life at 4°C. Standards are provided in a 0.9% NaCl, 0.05% NaN_3 solution.

1x Dye Reagent: 1 L of dye solution containing methanol and phosphoric acid. One bottle of dye reagent is sufficient for 200 assays using the standard 5 ml procedure or 4,000 assays using the microplate procedure.

BSA Standard, 2 mg/ml: Provided in 2 ml tubes.

Bovine Gamma-Globulin Standard, 2 mg/ml: Provided in 2 ml tubes.

Bovine Serum Albumin Standard Set:
Set of 7 concentrations of BSA (2, 1.5, 1, 0.75, 0.5, 0.25, 0.125 mg/ml) in 2 ml tubes.

Bovine Gamma-Globulin Standard Set:
Set of 7 concentrations of gamma-globulin (2, 1.5, 1, 0.75, 0.5, 0.25, 0.125 mg/ml) in 2 ml tubes.

Section 2
Instructions

2.1 Standard Protocol

1. The standard protocol can be performed in three different formats, 5 ml and a 1 ml cuvette assay, and a 250 µl microplate assay. The linear range of these assays for BSA is 125–1,000 µg/ml, whereas with gamma-globulin the linear range is 125–1,500 µg/ml.

2. Remove the 1x dye reagent from 4°C storage and let it warm to ambient temperature. Invert the 1x dye reagent a few times before use.

3. If 2 mg/ml BSA or 2 mg/ml gamma-globulin standard is used, refer to the tables in the

appendix as a guide for diluting the protein standard. (The dilutions in the tables are enough for performing triplicate measurements of the standards.) For the diluent, use the same buffer as in the samples (refer to Troubleshooting section for more information). Protein solutions are normally assayed in duplicate or triplicate. For convenience, the BSA or gamma-globulin standard sets can be used, but blank samples (0 µg/ml) should be made using water and dye reagent.

4. Pipet each standard and unknown sample solution into separate clean test tubes or microplate wells (the 1 ml assay may be performed in disposable cuvettes). Add the

1x dye reagent to each tube (or cuvette) and vortex (or invert). For microplates, mix the samples using a microplate mixer. Alternatively, use a multichannel pipet to dispense the 1x dye reagent. Depress the plunger repeatedly to mix the sample and reagent in the wells. Replace with clean tips and add reagent to the next set of wells.

Assay	Volume of Standard and Sample	Volume of 1x Dye Reagent
5 ml	100 µl	5 ml
1 ml	20 µl	1 ml
Microplate	5 µl	250 µl

5. Incubate at room temperature for at least 5 min. Samples should not be incubated longer than 1 hr at room temperature.

6. Set the spectrophotometer to 595 nm. Zero the instrument with the blank sample (not required for microplate readers). Measure the absorbance of the standards and unknown samples. Refer to Section 3 for data analysis.

   Note: If the spectrophotometer has a reference and sample holder, the instrument can be zeroed with two blank samples. If the effect of buffer on absorbance is required, zero the instrument with a cuvette filled with water and dye reagent in the reference holder.

2.2 Microassay Protocol

1. The microassay protocol can be performed in two different formats, a 2 ml cuvette assay

and a 300 µl microplate assay. The linear range of these assays for BSA is 1.25–10 µg/ml, whereas with gamma-globulin the linear range is 1.25–20 µg/ml.

2. Remove the 1x dye reagent from the 4°C storage and let it warm to ambient temperature. Invert the 1x dye reagent a few times before use.

3. Depending on the type of standard used, refer to the tables in the appendix as a guide for diluting the protein standard. For the diluent, use the same buffer as in the samples. Protein solutions are normally assayed in duplicate or triplicate. The dilutions in the tables provide enough volume to run triplicates.

4. Pipet each standard and unknown sample solution into separate clean test tubes, disposable cuvettes, or microplate wells. Add 1x dye reagent to each tube or cuvette and vortex: for microplates, mix the samples using a microplate mixer. Alternatively, use a multichannel pipet to dispense the 1x dye reagent. Depress and release the plunger repeatedly to mix the sample and reagent in the wells. Replace with clean tips and add reagent to the next set of wells.

Assay	**Volume of Standard and Sample**	**Volume of 1x Dye Reagent**
2 ml	1 ml	1 ml
Microplate	150 µl	150 µl

5. Incubate at room temperature for at least 5 min. Samples should not be incubated longer than 1 hr at room temperature.

6. Set the spectrophotometer to 595 nm. Zero the instrument with the blank sample (not required for microplate readers). Measure the absorbance of the standards, blanks, and unknown samples. Refer to Section 3 for data analysis.

   Note: If the spectrophotometer has a reference and sample holder, the instrument can be zeroed with the blank samples. If the effect of buffer on absorbance is required, zero the instrument with a cuvette filled with water and dye reagent in the reference holder.

Section 3
Data Analysis

1. If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.

2. Create a standard curve by plotting the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis). Determine the unknown sample concentration using the standard curve. If the samples were diluted, adjust the final concentration of the unknown samples by multiplying by the dilution factor used.

3. The microplate procedure may yield lower values than the standard and microassay procedures due to a shorter light path used in the microplate reader. This may decrease the level of detection of the assay.

4. Standard curve examples for the standard 5 ml procedure and the microassay procedure are listed in Figures 1 and 2, respectively.

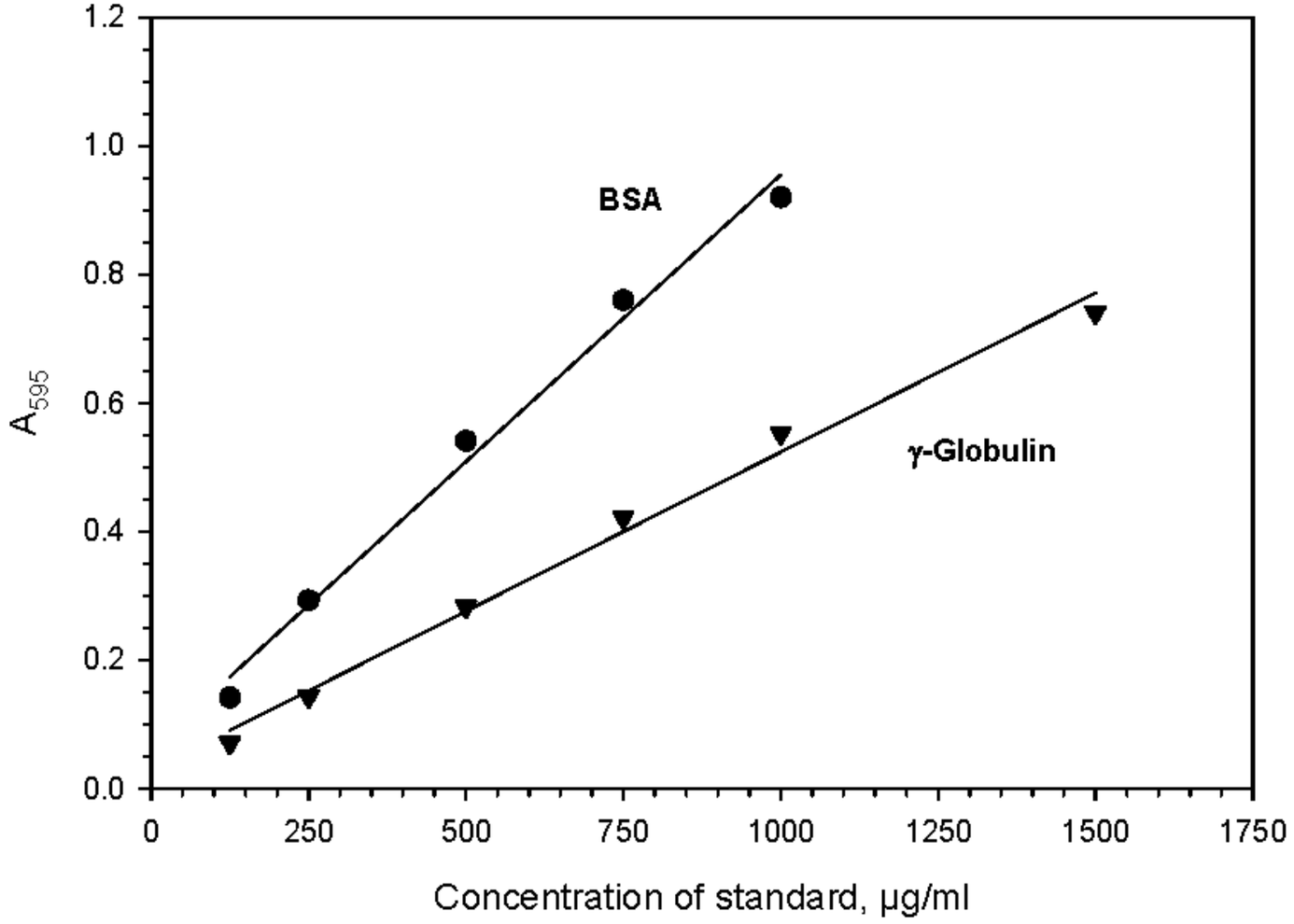

Fig 1. Typical standard curves using the standard 5 ml procedure with BSA and gamma-globulin standards.

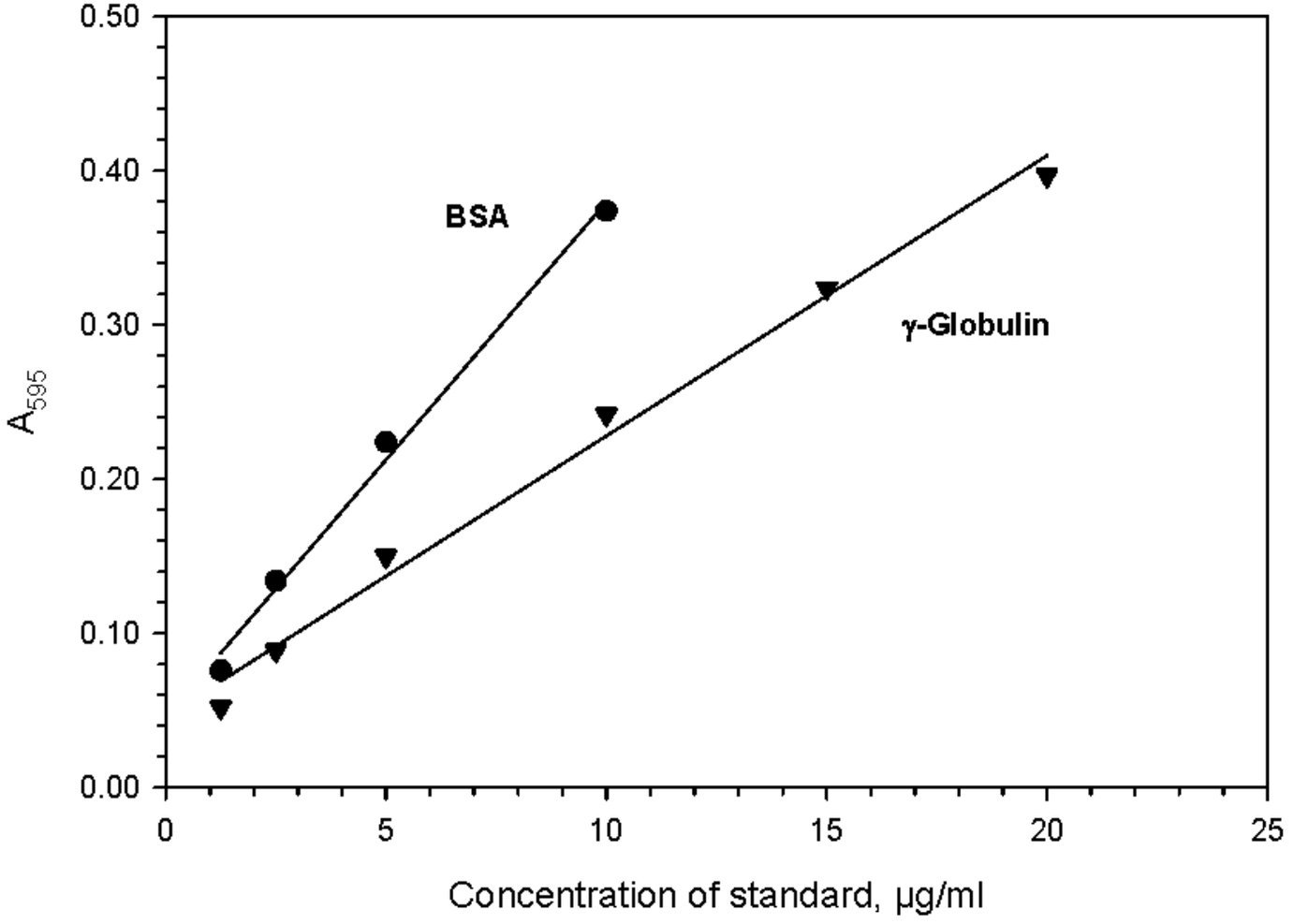

Fig 2. Typical standard curves using the microassay procedure with BSA and gamma-globulin standards.

Section 4
FAQs and Troubleshooting

Questions	Recommendations
1 The buffer that I normally use is not in the list of compatible reagents. How will I know if it interferes with the Quick Start Bradford assay?	It is best to run two standard curves, one with protein in the same buffer as your sample and one with protein in water, and then plot a graph of protein concentration versus absorbance. If the buffer does not interfere, the two standard curves will have identical slopes. Adding the buffer or interfering component to the standards used to construct the standard curve for the actual protein assay can compensate for partial interference.

2	My sample contains a detergent concentration that is incompatible with the Quick Start Bradford assay. How can I assay for protein?	If the protein concentration is high enough, a sample with detergent can be diluted so that the concentration of detergent is reduced to 0.1% or less. Alternatively, the Bio-Rad *DC*™ (detergent compatible) protein assay can be used (catalog #500-0111). The *DC* protein assay is a modified Lowry assay, which works in the presence of 1% ionic or nonionic detergent.
3	Is any sample preparation required?	In general, no. However, the protein must be solubilized. The sample cannot be a suspension or an unfiltered homogenate.

4 Does the binding of the blue dye to cuvettes skew results?

Bio-Rad's disposable polystyrene cuvettes (catalog #223-9950) are recommended for the protein assay. When using quartz cuvettes, the amount of dye that binds to them is negligible (Bradford 1976). Therefore, removal of the residual blue color between each sample reading is unnecessary. However, since the cuvettes may be used for subsequent procedures, there are several recommended treatments for dye removal:

- Rinse the cuvette with methanol, or
- Rinse the cuvette with glassware detergent, followed by ddi water, or
- Soak the cuvette in 0.1 N HCl for a few hours, then wash with ddi water.

5	May I use a wavelength other than 595 nm?	Yes. Absorbance can be measured at 580–610 nm.
6	Precipitation occurs in the tubes.	The samples contain a detergent in the buffer. Dilute the sample to reduce the detergent level or dialyze the samples.
7	Absorbance of protein standard and samples is very low.	The 1x dye reagent may be cold from 4°C storage. Warm the dye reagent to ambient temperature. The 1x dye reagent may be old. If it is over 1 year old, replace the dye reagent.
8	Absorbance of standard is acceptable, but absorbance of samples is very low.	The samples may contain a substance that interferes with the reaction, such as detergent or basic solutions. Check the compatibility guide (Table 1). Dilute the sample. Ensure the standards are diluted in the same buffer as the samples. The molecular weight of the sample protein may be low. The lower limit of detection for this method is approximately 3,000–5,000 daltons.

Section 5
Ordering Information

Catalog #	Description
500-0201	Quick Start Bradford Protein Assay Kit 1, includes 1 L 1x dye reagent and 5 x 2 ml bovine serum albumin standard at 2 mg/ml
500-0202	Quick Start Bradford Protein Assay Kit 2, includes 1 L 1x dye reagent and 5 x 2 ml bovine gamma globulin standard at 2 mg/ml
500-0203	Quick Start Bradford Protein Assay Kit 3, includes 1 L 1x dye reagent and bovine serum albumin standard set with 2 x 2 ml each concentration
500-0204	Quick Start Bradford Protein Assay Kit 4, includes 1 L 1x dye reagent and bovine gamma globulin standard set with 2 x 2 ml each concentration
500-0205	Quick Start Bradford 1 x Dye Reagent, 1L
500-0206	Quick Start Bovine Serum Albumin Standard, 2 mg/ml, 5 x 2 ml tubes

Catalog #	Description
500-0207	Quick Start Bovine Serum Albumin Standard Set, 2 x 2 ml each concentration, (2, 1.5, 1, 0.75, 0.5, 0.25, and 0.125 mg/ml)
500-0208	Quick Start Bovine Gamma Globulin Standard, 2 mg/ml, 5 x 2 ml tubes
500-0209	Quick Start Bovine Gamma Globulin Standard Set, 2 x 2 ml each concentration (2, 1.5, 1, 0.75, 0.5, 0.25, and 0.125 mg/ml)
223-9950	Disposable Polystyrene Cuvettes, 3.5 ml, box of 100
223-9955	Disposable Polystyrene Cuvettes, 1.5 ml, box of 100
224-0096	Costar 96-well Flat-Bottom ElA Plate, polystyrene, 5 per package, box of 100

Section 6
References

Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem, 72, 248–254 (1976)

Compton SJ and Jones CG, Mechanism of dye response and interference in the Bradford protein assay, Anal Biochem 151, 369–374 (1985)

Fanger B, Adaptation of the Bradford protein assay to membrane-bound proteins by solubilizing in glucopyranoside detergents, Anal Biochem 162, 11–17 (1987)

Fazekas de St. Groth S et al., Two new staining procedures for quantitative estimation of proteins on electrophoretic strips, Biochim Biophys Acta 71, 377–391 (1963)

Reisner AH et al., The use of Coomassie Brilliant Blue G-250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels, Anal Biochem 64, 509–516 (1975)

Sedmak JJ and Grossberg SE, A rapid, sensitive and versatile assay for protein using Coomassie Brilliant Blue G-250, Anal Biochem 79, 544–552 (1977)

Spector T, Refinement of the Coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 micrograms of protein, Anal Biochem 86, 142–146 (1978)

Section 7
Appendix

5 ml Standard Assay

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	300	2 mg/ml stock	0	2,000
2	375	2 mg/ml stock	125	1,500
3	325	2 mg/ml stock	325	1,000
4	175	Tube 2	175	750
5	325	Tube 3	325	500
6	325	Tube 5	325	250
7	325	Tube 6	325	125
8 (blank)	–	–	325	0

1 ml Standard Assay

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	70	2 mg/ml stock	0	2,000
2	75	2 mg/ml stock	25	1,500
3	70	2 mg/ml stock	70	1,000
4	35	Tube 2	35	750
5	70	Tube 3	70	500
6	70	Tube 5	70	250
7	70	Tube 6	70	125
8 (blank)	–	–	70	0

Microplate Standard Assay

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	20	2 mg/ml stock	0	2,000
2	30	2 mg/ml stock	10	1,500
3	20	2 mg/ml stock	20	1,000
4	20	Tube 2	20	750
5	20	Tube 3	20	500
6	20	Tube 5	20	250
7	20	Tube 6	20	125
8 (blank)	–	–	20	0

2 ml Microassay Cuvette Standard Dilutions

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	40	2 mg/ml stock	3,160	25
2	65	2 mg/ml stock	6,435	20
3	30	2 mg/ml stock	3,970	15
4	3,250	Tube 2	3,250	10
5	3,250	Tube 4	3,250	5
6	3,250	Tube 5	3,250	2.5
7	3,000	Tube 6	3,000	1.25
8 (blank)	–	–	3,200	0

2 ml Microassay Cuvette Dilutions for BSA or Gamma-Gobulin Standard Sets

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	40	2 mg/ml	3,160	25
2	35	2 mg/ml	3,465	20
3	35	1.5 mg/ml	3,465	15
4	35	1 mg/ml	3,465	10
5	35	0.5 mg/ml	3,465	5
6	35	0.25 mg/ml	3,465	2.5
7	35	0.125 mg/ml	3,465	1.25
8 (blank)	–	–	3,200	0

Microplate Microassay Dilutions for 2 mg/ml BSA or Gamma-Globulin

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	10	2 mg/ml stock	790	25
2	10	2 mg/ml stock	990	20
3	6	2 mg/ml stock	794	15
4	500	Tube 2	500	10
5	500	Tube 4	500	5
6	500	Tube 5	500	2.5
7	500	Tube 6	500	1.25
8 (blank)	–	–	500	0

Microplate Microassay Dilutions for BSA or Gamma-Globulin Standard Sets

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	10	2 mg/ml	790	25
2	5	2 mg/ml	495	20
3	5	1.5 mg/ml	495	15
4	5	1 mg/ml	495	10
5	5	0.5 mg/ml	495	5
6	5	0.25 mg/ml	495	2.5
7	5	0.125 mg/ml	495	1.25
8 (blank)	–	–	500	0

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Dr.
Hercules, CA 94547 USA
(510) 741-1000

1-800-424-6723 US only

4110065 Rev A