

# Sample Quantitation

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### **Protein Assay Kits**

#### See Also

SmartSpec Plus spectrophotometer: page 23. Microplate readers: page 330. Cuvettes: page 25. Determining the concentration of protein samples is critical for many experiments, and most protein samples can be quantitated by a colorimetric assay. In a typical protein assay, a chemical reagent is added to a protein sample solution, producing a color change that is measured with a spectrophotometer or microplate reader and compared to a standard curve of known protein concentrations. Bio-Rad offers four protein assays, each with a unique set of advantages. All assays can be easily automated for large numbers of samples.

Bio-Rad offers four protein assays:

- Quick Start<sup>™</sup> Bradford protein assay the single-step method for determining protein concentration in solution
- Bio-Rad protein assay for quantitating most proteins and polypeptides with MWs >3,000
- DC<sup>™</sup> protein assay for use with samples containing detergent
- RC DC<sup>™</sup> protein assay for complex sample solutions containing reducing agents and detergents

#### For More Information

Web: bio-rad.com/proteinassays Request or download bulletin: 1069

#### Protein Assay Selection Guide\*

	Quick Start Bradford	Bio-Rad	DC	RC DC
Application	Single-step method	Quantitates most proteins	For samples with detergent	For complex samples with reductants and detergents
Method adapted from	Bradford (1976)	Bradford (1976)	Lowry et al. (1951)	Lowry et al. (1951)
Standard-concentration assay Sample volume Linear range	100 µl 0.125–1.5 mg/ml	100 µl 0.125–1.5 mg/ml	100 µl 0.125–1.5 mg/ml	100 µl 0.2–1.5 mg/ml
Low-concentration assay Sample volume Linear range	1 ml 1.25–25 μg/ml	800 µl 1.25–25 µg/ml	200 μl 5–250 μg/ml	200 µl 5–250 µg/ml
Microplate assay sample volume	5 µl	10 μΙ	5 µl	**
Minimum incubation time	5 min	5 min	15 min	15 min
Assay wavelength	595 nm	595 nm	650-750 nm	650–750 nm

Standard-concentration assay is designed for smaller sample volumes of higher protein concentration, while the low-concentration assay is designed for larger sample volumes of lower protein concentration.

#### Quick Start™ Bradford Protein Assay

The Quick Start Bradford protein assay is a streamlined, accurate procedure for determining the concentration of protein in solution. Ready-to-use dye reagent and prediluted protein standards allow one-step determination of protein concentration.

Quick Start Bradford kits offer either bovine serum albumin or bovine  $\gamma$ -globulin standard sets. Each kit contains two aliquots each of seven concentrations (0.125, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 mg/ml) conveniently packaged in screwcap vials, eliminating ampoules and ensuring protein stability for one year when stored at 4°C. Standards are also available in five 2 mg/ml aliquots for creating your own dilutions.

The 1x dye reagent can be used for performing 1 and 5 ml standard assays, microplate assays, or microassays.



For More Information
Web: bio-rad.com/quickstart
Request or download bulletins: 1069 and 2969

<sup>\*\*</sup>To adapt the *RC DC* assay to a microplate format, follow the micro test tube (microfuge tube) assay protocol in the *RC DC* protein assay instruction manual up to the centrifugation step where the supernatant is discarded. The pellet can then be transferred to the microplate, and the microplate assay protocol in the *DC* protein assay instruction manual can be followed.

Ordering Inf	Ordering Information		
Catalog #	Description		
5000201	Quick Start Bradford Protein Assay Kit 1, includes 1x dye reagent (1 L), bovine serum albumin standard		
	(5 x 2 mg/ml); sufficient for 200 standard assays or 4,000 microplate assays		
5000202	Quick Start Bradford Protein Assay Kit 2, includes 1x dye reagent (1 L), bovine serum albumin standard set (2 sets of 7 concentration standards, 0.125–2.0 mg/ml, 2 ml)		
5000203	Quick Start Bradford Protein Assay Kit 3, includes 1x dye reagent (1 L), bovine γ-globulin standard		
	(5 x 2 mg/ml)		
5000204	Quick Start Bradford Protein Assay Kit 4, includes 1x dye reagent (1 L), bovine γ-globulin standard set		
	(2 sets of 7 concentration standards, 0.125–2.0 mg/ml, 2 ml)		
Reagents			
5000205	Quick Start Bradford 1x Dye Reagent, 1 L		
Accessories			
5000206	Quick Start Bovine Serum Albumin Standard, $5 \times 2$ ml vials of 2 mg/ml		
5000207	Quick Start Bovine Serum Albumin Standard Set, 2 sets of 7 concentration standards, 0.125–2.0 mg/ml		
5000208	Quick Start Bovine $\gamma$ -Globulin Standard, $5 \times 2$ ml vials of $2$ mg/ml		
5000209	Quick Start Bovine γ-Globulin Standard Set, 2 sets of 7 concentration standards, 0.125–2.0 mg/ml		

#### **Bio-Rad Protein Assay**

The Bio-Rad protein assay is a simple colorimetric assay for determining total protein concentration. It is easy to adapt the assay from the standard-concentration range to a low-concentration microassay or for use in 96-well microplates for rapid determinations.

The Bio-Rad protein assay is based on the Bradford dyebinding procedure (Bradford 1976), which measures the color change of Coomassie Brilliant Blue G-250 dye when it binds to protein (primarily to basic and aromatic amino acid residues). The assay quantitates most proteins and polypeptides with MWs >3,000. Some detergents and basic buffers interfere with the assay.

#### For More Information

Web: bio-rad.com/BRprotein

Request or download bulletins: 1069 and 1123



#### **Ordering Information** Catalog # 5000001 Bio-Rad Protein Assay Kit I, includes 450 ml dye reagent concentrate, bovine γ-globulin standard; sufficient for 440 standard assays or 2,200 microplate assays 5000002 Bio-Rad Protein Assay Kit II, includes 450 ml dye reagent concentrate, bovine serum albumin standard; sufficient for 440 standard assays or 2,200 microplate assays Reagents 5000006 Bio-Rad Protein Assay Dye Reagent Concentrate, 450 ml Accessories 5000005 Protein Standard I, bovine γ-globulin, reconstituted volume 20 ml 5000007 Protein Standard II, bovine serum albumin, reconstituted volume 20 ml

#### **DC**<sup>™</sup> Protein Assay

The DC (detergent compatible) protein assay is a colorimetric assay for protein determination of samples that contain detergents. The reaction is similar to the well-documented Lowry assay (Lowry et al. 1951), but has been modified to save time. The DC protein assay requires only a single 15 minute incubation, and the absorbance readings are stable for at least 2 hours.

#### For More Information

Web: bio-rad.com/DCprotein

Request or download bulletins: 1069, 1731, 1770, and 1909



Catalog #	Description	
5000111	DC Protein Assay Kit I, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent,	
	5 ml surfactant solution, bovine $\gamma$ -globulin standard; sufficient for 450 standard assays	
5000112	DC Protein Assay Kit II, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent,	
	5 ml surfactant solution, bovine serum albumin standard; sufficient for 450 standard assays	
Reagents		
5000113	Protein Assay Reagent A, 250 ml, alkaline copper tartrate solution	
5000114	Protein Assay Reagent B, 1 L, dilute Folin reagent	
5000115	Protein Assay Reagent S, 5 ml, surfactant solution	
5000116	DC Protein Assay Reagents Package, includes 250 ml alkaline copper tartrate solution,	
	2 L dilute Folin reagent, 5 ml surfactant solution; sufficient for 450 standard assays	
Accessories		
5000005	Protein Standard I, bovine γ-globulin, lyophilized (reconstituted volume 20 ml)	
5000007	Protein Standard II, bovine serum albumin, lyophilized (reconstituted volume 20 ml)	

#### See Also

Protein sample preparation: page 2. Cuvettes: page 25.

#### RC DC<sup>™</sup> Protein Assay

The RC DC (reducing agent and detergent compatible) protein assay is a colorimetric assay for protein determination in the presence of reducing agents and detergents. The RC DC protein assay, based on the Lowry protocol (Lowry et al. 1951), includes the features of the original DC protein assay. Its compatibility with a broader range of reagents allows simplified protein quantitation directly in complex sample solutions.

For More Information

Web: bio-rad.com/RCDCprotein

Request or download bulletins: 1069 and 2610



Ordering Information		
Catalog #	Description	
5000121	RC DC Protein Assay Kit I, includes RC reagents package, DC protein assay reagents package,	
	bovine $\gamma$ -globulin standard; sufficient for 450 standard assays	
5000122	RC DC Protein Assay Kit II, includes RC reagents package, DC protein assay reagents package,	
	bovine serum albumin standard; sufficient for 450 standard assays	
Reagents		
5000113	Protein Assay Reagent A, 250 ml, alkaline copper tartrate solution	
5000114	Protein Assay Reagent B, 1 L, dilute Folin reagent	
5000115	Protein Assay Reagent S, 5 ml, surfactant solution	
5000116	DC Protein Assay Reagents Package, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin	
	reagent, 5 ml surfactant solution; sufficient for 450 standard assays	
5000120	RC DC Protein Assay Reagents Package, includes RC reagents package and DC protein assay reagents	
	package; sufficient for 450 standard assays	
5000119	RC Reagents Package, includes RC reagent I (250 ml) and RC reagent II (250 ml); sufficient for	
	500 standard assays	
5000117	RC Reagent I, 250 ml	
5000118	RC Reagent II, 250 ml	
Accessories		
5000005	Protein Standard I, bovine \( \gamma \)-globulin, lyophilized (reconstituted volume 20 ml)	
5000007	Protein Standard II, bovine serum albumin, lyophilized (reconstituted volume 20 ml)	

### Spectrophotometry

#### SmartSpec<sup>™</sup> Plus Spectrophotometer

The SmartSpec Plus spectrophotometer has a more complete range of features and functions than many other benchtop spectrophotometers, offering affordable performance, stability, and functionality.

The SmartSpec Plus is a UV/visible spectrophotometer with a working wavelength range of 200-800 nm. For routine work with nucleic acid and protein samples, the SmartSpec Plus spectrophotometer provides you with an instrument for:

- Quantitation of DNA, RNA, oligonucleotides, and proteins
- Monitoring cell culture growth
- Simple kinetics assays
- Wavelength scans with peak detection

A simple, menu-driven interface simplifies assays and provides common sample computations at the touch of a button. Conversion factors can be stored and modified. The SmartSpec Plus instrument can automatically provide results such as:

- A<sub>260</sub>/A<sub>280</sub> ratio for nucleic acid purity
- · Quantitation that takes dilution factors into account



- Sample concentration in µg/ml (additionally in pmol/µl for oligonucleotides)
- Molar extinction coefficient and MW of oligonucleotides

At the end of an assay, a report can be printed that shows the user, date, and results.

Protein assay kits: page 20. Cuvettes: page 25.

#### See Also

Protein assay kits: page 20. SmartSpec Plus spectrophotometer: page 23.

#### **Nucleic Acid Quantitation**

The SmartSpec Plus spectrophotometer offers a complete solution for the quantitation of dsDNA, ssDNA, or RNA by either using the preprogrammed conversion factors or entering a value that is best for the sample being assayed. The SmartSpec Plus instrument will provide absorbance, concentration, and purity values so you can proceed confidently with downstream experiments.

#### **Protein Quantitation**

The SmartSpec Plus instrument has preprogrammed methods for the quantitation of proteins by the Bradford, Lowry, and BCA methods. Features built into each assay method facilitate data collection and present a complete analysis of assay results.

- Standards can be analyzed in groups of up to nine replicates
- Up to 10 standard curves can be stored under user-assigned names
- Mean and standard deviation values are automatically calculated for each replicate group
- Printable report includes a standard curve with r<sup>2</sup> value

#### **Other Benefits**

- Built-in thermal printer
- Xenon flash lamp extends lamp life and reduces maintenance costs
- User interface enables choice of six different languages: English, French, German, Italian, Japanese, and Spanish
- Compact, space-saving design

#### Cuvettes

Bio-Rad offers compatible quartz and UV-transparent plastic cuvettes.

#### For More Information

Web: bio-rad.com/spectrophotometry Request or download bulletin: 2826

\* This product may not be available in all regions. Please check availability with your Bio-Rad Sales Representative.

#### Spectrophotometer Cuvette Selection Guide

Min. Volume, µl	Max. Volume, μl	Cuvette Type	Pathlength, mm	Catalog #
1,000	3,500	Standard cuvette, quartz	10	1702502
500	1,400	Semimicrovolume cuvette, quartz	10	1702503
200	700	Microvolume cuvette, quartz	10	1702504
80	100	Submicrovolume cuvette, quartz	10	1702505
50	1,500	trUView™ cuvettes	10	1702510, 1702511

#### **Ordering Information** Catalog # Description SmartSpec Plus Spectrophotometer 1702525 SmartSpec Plus Spectrophotometer Accessories 1702502 Standard Cuvette, 1-3.5 ml, quartz 1702503 Semimicrovolume Cuvette, 0.5-1.4 ml, quartz 1702504 Microvolume Cuvette, 200-700 µl, quartz 1702505 Submicrovolume Cuvette, 80-100 µl, quartz 1702506 SmartSpec Printer Paper, 5 pack 1702507 Spring, cuvette holder

### **Cuvettes**

#### trUView<sup>™</sup> Cuvettes

These disposable cuvettes are suitable for most UV and visible spectroscopic assays. Highly transparent trUView cuvettes allow accurate and precise quantitation of DNA, RNA, and protein. Individually packaged trUView cuvettes are free of contaminants and guaranteed DNase, RNase, and pyrogen free. Features of trUView cuvettes include:

- Low volume requirement (≥50 µl) that conserves limited samples
- Individual packaging to prevent scratching and contamination
- Up to 70% light transmission at 260 nm, ensuring accurate nucleic acid quantitation

#### trUView vs. Quartz Cuvettes

trUView cuvettes provide several advantages over traditional quartz cuvettes for quantitative UV applications, having characteristics that can supplement or replace the use of quartz cuvettes. trUView cuvettes are:

- Ready to use without lengthy sterilization procedures
- Disposable and less expensive to replace

#### For More Information

Web: bio-rad.com/spectrophotometry



Catalog #	Description
1702510	trUView Cuvettes, pack of 50
1702511	trUView Cuvettes, pack of 100



#### **Cuvettes**

#### See Also

Protein assay kits: page 20. SmartSpec Plus spectrophotometer: page 23.

#### **Standard and Semimicrovolume Cuvettes**

Standard 3.5 ml cuvettes are ideal for use with protein assays (pags 20-22). They adsorb less Coomassie Blue dye than glass or quartz cuvettes. Assays can be mixed directly in the cuvette. The volume of reagents can be reduced, yielding more assays per kit. Semimicrovolume 1.5 ml cuvettes are ideal for precise quantitation of small volume samples. Both standard and semimicrovolume cuvettes are sized to fit most spectrophotometers. The cuvettes offer smooth optical surfaces for consistent and accurate readings.

Bio-Rad also offers other cuvettes for specialized applications, including quartz and disposable cuvettes for SmartSpec<sup>™</sup> spectrophotometers and disposable trUView<sup>™</sup> cuvettes for precise quantitation of small samples of DNA, RNA, and proteins.

For More Information

Web: bio-rad.com/spectrophotometry



Ordering Information		
Catalog #	Description	
Disposable	Polystyrene Cuvettes	
2239950	Standard Disposable Polystyrene Cuvettes, 3.5 ml, 100	
2239955	Semimicrovolume Disposable Polystyrene Cuvettes, 1.5 ml, 100	
Quartz Cuve	ettes	
1702502	Standard Cuvette, 1-3.5 ml, quartz, for use with the SmartSpec Plus spectrophotometer	
1702503	Semimicrovolume Cuvette, 0.5-1.4 ml, quartz, for use with the SmartSpec Plus spectrophotometer	
1702504	Microvolume Cuvette, 200–700 μl, quartz, for use with the SmartSpec Plus spectrophotometer	
1702505	Submicrovolume Cuvette, 80-100 µl, quartz, for use with the SmartSpec Plus spectrophotometer	
VersaFluor [	Disposable Cuvettes and Cuvette Racks	
1702415	Standard Cuvettes, 12.5 x 12.5 mm (outside dimensions), 4-sided, optically clear polystyrene, 3.5 ml, 100	
1702416	Microcuvettes, 3.0 x 3.0 mm (outside dimensions), 4-sided, optically clear polystyrene, 150–350 µl, 100	
1660485	Cuvette Racks, hold 12 cuvettes each, set of 5	

### **Sample Quantitation**

**Subject Index** 

## Subject Index

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