Sample Quantitation

Spectrophotometry

SmartSpec™ Plus Spectrophotometer

Order Info: Pg 23

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_{260}/A_{280} 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.

Nucleic Acid Quantitation

The SmartSpec Plus spectrophotometer offers a complete solution for the quantitation of dsDNA, ssDNA, or RNA by using either 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 your 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 9 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^2 value

Other Benefits

- Built-in thermal printer
- Xenon flash lamp extends lamp life and reduces maintenance costs
- User interface enables choice of 6 different languages: English, French, German, Italian, Japanese, and Spanish
- Compact, space-saving design

For More Information
Web: www.bio-rad.com/spectrophotometry
Request or download bulletin: 2826

Cuvettes

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

Spectrophotometer Cuvette Selection Guide

Min. Volume, µl	Max. Volume, µl	Cuvette Type	Pathlength, mm	Catalog #
1,000	3,500	Standard cuvette, quartz	10	170-2502
500	1,400	Semimicrovolume cuvette, quartz	10	170-2503
200	700	Microvolume cuvette, quartz	10	170-2504
80	100	Submicrovolume cuvette, quartz	10	170-2505
50	1,500	trUView™ cuvettes	10	170-2510, 170-2511

trUView™ Cuvettes

Order Info: Pg 23

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 are 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, and have 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: www.bio-rad.com/spectrophotometry

Standard and Semimicrovolume Cuvettes

Order Info: Pg 23

Standard 3.5 ml cuvettes are ideal for use with protein assays (pages 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™ spectrophotometers and disposable trUView cuvettes for precise quantitation of small samples of DNA, RNA, and proteins.

For More Information

Web: www.bio-rad.com/spectrophotometry

Protein Assays

See Also

SmartSpec Plus spectrophotometer: page 18.

Microplate readers: page 278.

Cuvettes: page 19.

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™ 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*™ protein assay** — for use with samples containing detergent
- ***RC DC*™ protein assay** — for complex sample solutions containing reducing agents and detergents

For More Information
Web: www.bio-rad.com/proteinassays
Request or download bulletin: 1069

Protein Assay Selection Guide*

	Quick Start Bradford	Bio-Rad	*DC*	*RC DC*
Method adapted from	Bradford (1976)	Bradford (1976)	Lowry et al. (1951)	Lowry et al. (1951)
Standard-concentration assay				
Sample volume	100 µl	100 µl	100 µl	100 µl
Linear range	0.125–1.5 mg/ml	0.125–1.5 mg/ml	0.125–1.5 mg/ml	0.2–1.5 mg/ml
Low-concentration assay				
Sample volume	1 ml	800 µl	200 µl	200 µl
Linear range	1.25–25 µg/ml	1.25–25 µg/ml	5–250 µg/ml	5–250 µg/ml
Microplate assay sample volume	5 µl	10 µl	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.

** 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.

Quick Start™ Bradford Protein Assay

Order Info: Pg 23

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 γ-globulin standard sets. Each kit contains two aliquots of seven concentrations (0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 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 ml and 5 ml standard assays, microplate assays, or microassays.

For More Information
Web: www.bio-rad.com/quickstart
Request or download bulletin: 1069

Bio-Rad Protein Assay

Order Info: Pg 23

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 dye-binding 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 molecular weights >3,000. Some detergents and basic buffers interfere with the assay.

For More Information
Web: www.bio-rad.com/BRprotein
Request or download bulletins: 1069 and 1123

DC™ Protein Assay

Order Info: Pg 24

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: www.bio-rad.com/DCprotein
Request or download bulletins: 1069 and 1909

See Also

Protein sample preparation: pages 7–12.

Cuvettes: page 19.

RC DC™ Protein Assay

Order Info: Pg 24

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: www.bio-rad.com/RCDCprotein
Request or download bulletins: 1069 and 2610

Spectrophotometry

Catalog #	Description
SmartSpec Plus Spectrophotometer	**Pg 18**
170-2525	**SmartSpec Plus Spectrophotometer**
Accessories	
170-2502	**Standard Cuvette**, 1–3.5 ml, quartz
170-2503	**Semimicrovolume Cuvette**, 0.5–1.4 ml, quartz
170-2504	**Microvolume Cuvette**, 200–700 µl, quartz
170-2505	**Submicrovolume Cuvette**, 80–100 µl, quartz
170-2506	**SmartSpec Printer Paper**, 5 pack
170-2507	**Spring**, cuvette holder
Standard and Semimicrovolume Cuvettes	**Pg 19**
Disposable Polystyrene Cuvettes	
223-9950	**Standard Disposable Polystyrene Cuvettes**, 3.5 ml, 100
223-9955	**Semimicrovolume Disposable Polystyrene Cuvettes**, 1.5 ml, 100
Quartz Cuvettes	
170-2502	**Standard Cuvette**, 1–3.5 ml, quartz, for use with the SmartSpec Plus spectrophotometer
170-2503	**Semimicrovolume Cuvette**, 0.5–1.4 ml, quartz, for use with the SmartSpec Plus spectrophotometer
170-2504	**Microvolume Cuvette**, 200–700 µl, quartz, for use with the SmartSpec Plus spectrophotometer
170-2505	**Submicrovolume Cuvette**, 80–100 µl, quartz, for use with the SmartSpec Plus spectrophotometer
trUView Cuvettes	
170-2510	**trUView Cuvettes**, pack of 50
170-2511	**trUView Cuvettes**, pack of 100
VersaFluor Disposable Cuvettes	
170-2415	**Standard Cuvettes**, 12.5 x 12.5 mm (outside dimensions), 4-sided, optically clear polystyrene, 3.5 ml, 100
170-2416	**Microcuvettes**, 3.0 x 3.0 mm (outside dimensions), 4-sided optically clear polystyrene, 150–350 µl, 100

Protein Assays

Quick Start Bradford Protein Assay	**Pg 21**
500-0201	**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
500-0202	**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)
500-0203	**Quick Start Bradford Protein Assay Kit 3**, includes 1x dye reagent (1 L), bovine γ-globulin standard (5 x 2 mg/ml)
500-0204	**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	
500-0205	**Quick Start Bradford 1x Dye Reagent**, 1 L
Accessories	
500-0206	**Quick Start Bovine Serum Albumin Standard**, 5 x 2 ml vials of 2 mg/ml
500-0207	**Quick Start Bovine Serum Albumin Standard Set**, 2 sets of 7 concentration standards, 0.125–2.0 mg/ml
500-0208	**Quick Start Bovine γ-Globulin Standard**, 5 x 2 ml vials of 2 mg/ml
500-0209	**Quick Start Bovine γ-Globulin Standard Set**, 2 sets of 7 concentration standards, 0.125–2.0 mg/ml
Bio-Rad Protein Assay	**Pg 21**
500-0001	**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
500-0002	**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	
500-0006	**Bio-Rad Protein Assay Dye Reagent Concentrate**, 450 ml
Accessories	
500-0005	**Protein Standard I**, bovine γ-globulin, reconstituted volume 20 ml
500-0007	**Protein Standard II**, bovine serum albumin, reconstituted volume 20 ml

Catalog #	Description
***DC* Protein Assay**	**Pg 22**
500-0111	***DC* Protein Assay Kit I**, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution, bovine γ-globulin standard; sufficient for 450 standard assays
500-0112	***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	
500-0113	**Protein Assay Reagent A**, 250 ml, alkaline copper tartrate solution
500-0114	**Protein Assay Reagent B**, 1 L, dilute Folin reagent
500-0115	**Protein Assay Reagent S**, 5 ml, surfactant solution
500-0116	***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	
500-0005	**Protein Standard I**, bovine γ-globulin, lyophilized (reconstituted volume 20 ml)
500-0007	**Protein Standard II**, bovine serum albumin, lyophilized (reconstituted volume 20 ml)
***RC DC* Protein Assay**	**Pg 22**
500-0121	***RC DC* Protein Assay Kit I**, includes *RC* reagents package, *DC* protein assay reagents package, bovine γ-globulin standard; sufficient for 450 standard assays
500-0122	***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	
500-0113	**Protein Assay Reagent A**, 250 ml, alkaline copper tartrate solution
500-0114	**Protein Assay Reagent B**, 1 L, dilute Folin reagent
500-0115	**Protein Assay Reagent S**, 5 ml, surfactant solution
500-0116	***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
500-0120	***RC DC* Protein Assay Reagents Package**, includes *RC* reagents package and *DC* protein assay reagents package; sufficient for 450 standard assays
500-0119	***RC* Reagents Package**, includes *RC* reagent I (250 ml) and *RC* reagent II (250 ml); sufficient for 500 standard assays
500-0117	***RC* Reagent I**, 250 ml
500-0118	***RC* Reagent II**, 250 ml
Accessories	
500-0005	**Protein Standard I**, bovine γ-globulin, lyophilized (reconstituted volume 20 ml)
500-0007	**Protein Standard II**, bovine serum albumin, lyophilized (reconstituted volume 20 ml)