Colorimetric Protein Assays

Introduction

Bio-Rad offers four colorimetric assays for protein quantitation: the Quick Start™ Bradford protein assay, the Bio-Rad protein assay for general use, the *DC*™ protein assay for samples solubilized in detergent, and the *RC DC*™ protein assay for samples in the presence of both reducing agents and detergents. The need for all types of assays is clear. The Quick Start Bradford protein assay comes with prediluted standards and 1x dye reagent for maximum convenience and ease of use. Both the Quick Start Bradford and Bio-Rad protein assays can be used to assay samples in common buffers, but are sensitive to many detergents present in concentrations greater than 0.1%; the *DC* protein assay can be used to assay protein in the presence of 1% detergent in addition to many common reagents; the *RC DC* protein assay is both reducing agent compatible (RC) and detergent compatible (DC). Consult Appendices A, B, C, and D for lists of compatible substances.

The table below describes features of each assay:

	Quick Start Bradford	Bio-Rad	*DC*	*RC DC*
Standard assay sample volume	100 µl	100 µl	100 µl	100 µl
Microplate assay sample volume	5 µl	10 µl	5 µl	*
Linear range for standard assay	0.125–1.5 mg/ml	0.2–1.5 mg/ml	0.2–1.5 mg/ml	0.2–1.5 mg/ml
Minimum incubation time	5 min	5 min	15 min	15 min
Adapted from method of	Bradford (1976)	Bradford (1976)	Lowry et al. (1951)	Lowry et al. (1951)

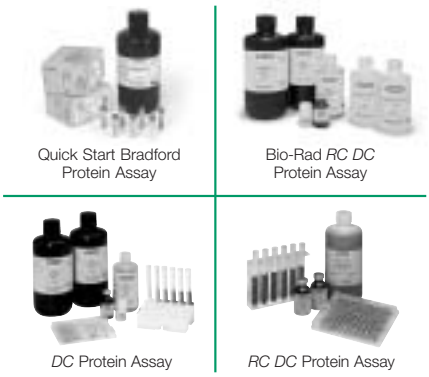
All assays are easy to use, require little reagent preparation, and give accurate and reproducible results.

* To adapt the *RC DC* assay to a microplate format, follow the micro test tube (microfuge tube) assay protocol in the *RC DC* 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 manual can be followed.

Principle

Quick Start Bradford and Bio-Rad Protein Assay

The Quick Start Bradford and Bio-Rad protein assays are based on an observed shift in the absorbance maximum when Coomassie Brilliant Blue G-250 dye reacts with protein (Fazekas de St. Groth et al. 1963, Reisner et al. 1975, Sedmak and Grossberg 1977). Bradford (1976) first demonstrated the usefulness of this principle in protein assays. Spector (1978) found that the extinction coefficient of a dye-albumin complex 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. Over a broad range of protein concentrations, the dye-binding method gives an accurate but not entirely linear response.



As with any colorimetric protein assay, there is variation in color response to different proteins. Both the color change due to dye binding and the variation in color response can be attributed to the dye having 3 absorbing species: a red cationic species, a green neutral species, and a blue anionic species. At the assay pH, the dye molecules are doubly protonated and are present as the red cationic dye form. Binding of the dye to protein stabilizes the blue anionic dye form, detected at 595 nm. Work with synthetic polyamino acids indicates that Coomassie Brilliant Blue G-250 binds primarily to basic (especially arginine) and aromatic amino acid residues (Compton and Jones 1985). Interference from nonprotein compounds is due to their ability to shift the equilibria among the three 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.

Thus, dye binding (and the associated color change that is the basis of the protein assay) requires a protein containing active basic or aromatic residues. Proteins larger than about 8 or 9 amino acid residues are the only substances known to be capable of this stabilization.

DC Protein Assay

The *DC* protein assay (patent pending) is a colorimetric assay for protein concentration following detergent solubilization. The reaction is similar to the well-documented Lowry assay (Lowry et al. 1951), but with the following improvements: the reaction reaches 95% of its maximum color development within 15 min, and the color changes less than 5% in 1 hr or 10% in 2 hr.

The assay is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. As with the Lowry assay, there are 2 steps which lead to color development: the reaction between protein and copper at alkaline pH, and the subsequent reduction of Folin reagent by the copper-treated protein. Color development is primarily due to the amino acids tyrosine and tryptophan, and to a lesser extent cystine, cysteine, and histidine present in the proteins. These amino acids reduce the Folin reagent, yielding several possible reduced species that have a characteristic blue color (with maximum absorbance at 750 nm and minimum absorbance at 405 nm) (Peterson 1979). Absorbance can be reliably measured between 650 and 750 nm.

Bio-Rad *RC DC* Protein Assay

The *RC DC* protein assay is a colorimetric assay for determining protein concentration in the presence of both reducing agents and detergents. The *RC DC* protein assay has all the unique capabilities of the original *DC* protein assay, in addition to covering a broader range of reagents. This assay is based on a modification of the Lowry protocol (Lowry et al. 1951). The *RC DC* protein assay is both reducing agent compatible (RC) and detergent compatible (DC) (Figure 1). Protein quantitation can be performed directly in complex mixtures including Laemmli buffer and ReadyPrep™ reagents. As with the *DC* protein assay, absorbance is measured at 750 nm. The absorbance values will be stable for at least 1 hr.

Selecting a Protein Standard

In any protein assay, the best 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. If a direct comparison of two different protein assays is being performed, the same standard should be used for both procedures. Bio-Rad offers two standards: bovine γ-globulin (standard I) and bovine serum albumin (standard II).

With the Quick Start Bradford and Bio-Rad protein assays, the dye color development is significantly greater with albumin than with most other proteins, including γ-globulin (Figure 2). Therefore, the albumin standard (standard II) would be appropriate if the sample contains primarily

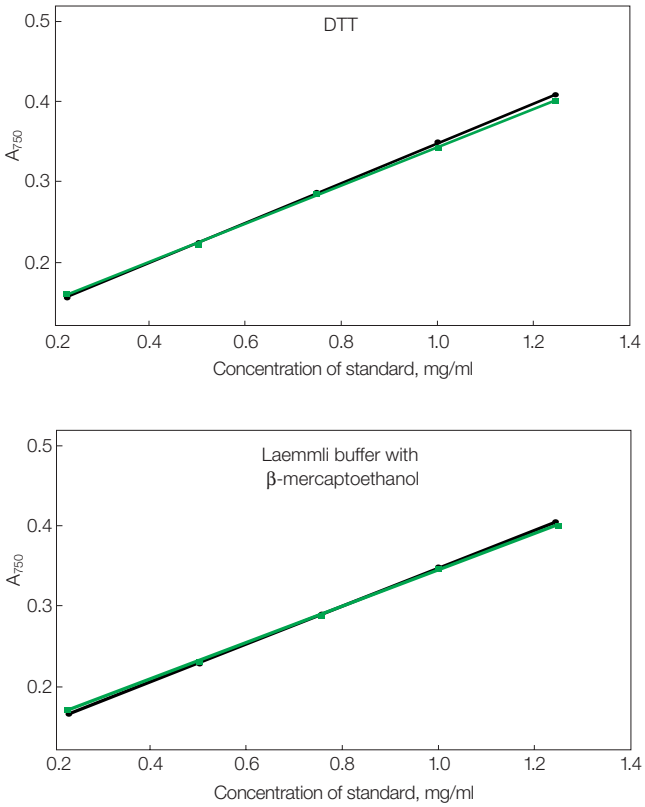


Fig. 1. Standard curves generated with carbonic anhydrase in water vs. in presence of reducing agents, using the *RC DC* protein assay. Top, 350 mM DTT; bottom, Laemmli buffer with 5% β-mercaptoethanol. Black, water; green, reducing agents. (Data points are averages for triplicates; error bars showing standard deviations are smaller than symbols.)

albumin, or if the protein being assayed gives a similar response to the dye. For a color response that is typical of many proteins, the γ-globulin standard is appropriate.

The *DC* and *RC DC* protein assays show little difference in color development between albumin and γ-globulin (Figure 3). It is recommended, however, that the same standard be used if comparisons are to be made between different assays.

Because each reaction is based on amino acid composition, different proteins will interact differently with the reagents of the Quick Start Bradford protein assay, the Bio-Rad protein assay, the *DC* protein assay, and the *RC DC* protein assay. For this reason, we recommend that for relative measurements of protein concentration, one standard be chosen.

Which Method Should I Choose?

Each assay has distinct advantages, depending upon the application. The Quick Start Bradford and Bio-Rad protein assays, both based on the Bradford method, can be used in the presence of sugars, dithiothreitol (DTT), and β-mercaptoethanol, which at higher concentrations interfere with the *DC* protein assay. Alternatively, the *DC* protein assay can be used in the presence of detergents or sodium hydroxide, 2 components known to interfere with the

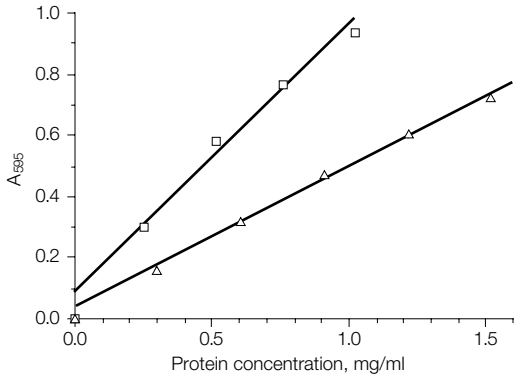


Fig. 2. Typical standard curves for the Bio-Rad protein assay: bovine serum albumin (□) and γ-globulin (△).

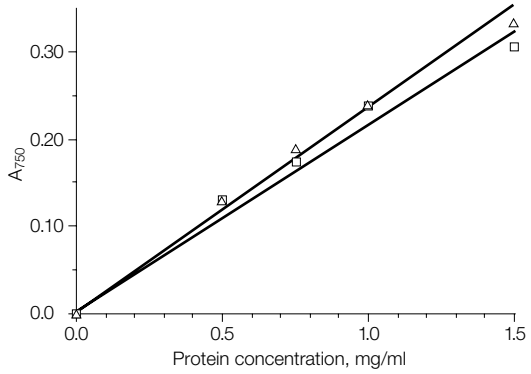


Fig. 3. Typical standard curves for the *DC* protein assay: bovine serum albumin (□) and γ-globulin (△).

Bradford assay. If the sample is contained in a buffer that is compatible with both assays, then either may be used. The *RC DC* protein assay is both reducing agent compatible s(RC) and detergent compatible (DC). This assay is ideal for protein quantitation in the presence of both reducing agents and detergents, protein quantitation in sample loading buffer prior to performing 1-D or 2-D gel electrophoresis, or quantitation of protein recovery in cell lysis buffer after harvest.

Interference

To ascertain if a particular reagent interferes with an assay, prepare a standard curve with protein diluted with the reagent in question. Run a side-by-side comparison of this standard curve with one that has been prepared with water. If a significant difference in dye color development is observed, correction for the interfering substance is recommended. In general, preparing the standard curve by diluting the standard protein with the sample buffer will yield the most accurate results. We have prepared an extensive list of compounds (Appendices A, B, C, and D) which are found to be compatible with each assay.

Assay Methods

It is considered good practice to run a calibration curve each time a protein assay is performed. To prepare a standard curve for each assay, make several dilutions of protein to cover the range specified for each assay procedure below. Note that microplate assays are about twice as sensitive as test tube assays.

To determine protein concentration of samples, plot a standard curve of absorbance vs. concentration of the protein standards first. Using the standard curve, determine the concentration of each sample from its absorbance by interpolation.

(For detailed protocols, consult the instruction manuals.)

Quick Start Bradford and Bio-Rad Protein Assay

Standard Assay (0.125–1.5 mg/ml)

1. Pipet 100 µl of standards or samples into clean, dry test tubes.
2. Add 5.0 ml of 1x dye reagent to each tube.
3. Vortex until homogeneous color forms (about 3 sec).
4. Let samples incubate at room temperature for 5–60 min, then measure absorbance at 595 nm.

Microassay (1.25–20 µg/ml)

1. Pipet 1.0 ml of standards or samples into clean, dry test tubes.
2. Add 1.0 ml of 1x dye reagent to each tube.
3. Vortex until homogeneous color forms (about 3 sec).
4. Let samples incubate at room temperature for 5–60 min, then measure absorbance at 595 nm.

Microplate Standard Assay (0.05–0.5 mg/ml)

1. Pipet 5 µl of standards or samples into wells.
2. Add 250 µl 1x dye reagent to wells.
3. Mix the samples thoroughly using a microplate mixer. Alternatively, use a multichannel pipet to dispense the reagent and depress the plunger repeatedly to mix the samples.
4. Incubate microplate at room temperature for 5–60 min, then measure absorbance at 595 nm.

Microplate Microassay (8–80 µg/ml)

1. Pipet 150 µl of standards or samples into wells.
2. Add 150 µl of 1x dye reagent into wells.
3. Mix the samples thoroughly as described above.
4. Incubate microplate at room temperature for 5–60 min, then measure absorbance at 595 nm.

DC Protein Assay

Standard Assay (0.2–1.5 mg/ml)

1. Pipet 100 µl of standards or samples into clean, dry test tubes.
2. Add 500 µl of working reagent A´, and vortex.
3. Add 4.0 ml of reagent B, and vortex.
4. Incubate at room temperature for a minimum of 15 min, then measure absorbance at 750 nm.

Microplate Assay (0.2–1.5 mg/ml)

1. Pipet 5 µl of standards or samples into wells.
2. Add 25 µl of working reagent A´ to each well. Gently mix the plate.
3. Mix the samples thoroughly using a microplate mixer. Alternatively, use a multichannel pipet to dispense the reagent and depress the plunger repeatedly to mix the samples.
4. Incubate microplate at room temperature for at least 15 min, then measure absorbance at 750 nm.

RC DC Protein Assay

Standard Assay (0.2–1.5 mg/ml)

1. Pipet 100 µl of standards and samples into clean, dry test tubes.
2. Add 500 µl *RC* reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
3. Add 500 µl *RC* reagent II into each tube and vortex. Centrifuge the tubes at 15,000 x g for 3–5 min.
4. Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
5. Add 510 µl reagent A´ to each tube and vortex. Incubate tubes at room temperature for 5 min, or until precipitate is completely dissolved. Vortex before proceeding to the next step.
6. Add 4 ml of *DC* reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.
7. After the 15 min incubation, absorbances can be read at 750 nm. The absorbances will be stable for at least 1 hr.

Micro Test Tube Assay (0.2–1.5 mg/ml)

1. Pipet 25 µl of standards and samples into clean, dry microfuge tubes.
2. Add 125 µl *RC* reagent I into each tube and vortex. Incubate the tubes for 1 minute at room temperature.
3. Add 125 µl *RC* reagent II into each tube and vortex. Centrifuge the tubes at 15,000 x g for 3–5 min.
4. Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
5. Add 127 µl reagent A´ to each microfuge tube and vortex. Incubate tubes at room temperature for 5 min, or until precipitate is completely dissolved. Vortex again before proceeding to next step.
6. Add 1 ml of *DC* reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.
7. After the 15 min incubation, absorbances can be read at 750 nm. The absorbances will be stable for at least 1 hr.

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Appendix A

Reagents Compatible With the Quick Start Bradford Protein Assay When Using the Standard Procedure

Acetone, 10%
Acetonitrile, 10%
Ammonium sulfate, 1 M
Ampholytes, pH 3–10, 0.5%
ASB-14, 0.025%
Ascorbic acid, 50 mM
Bis-Tris, pH 6.5, 0.2 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
HEPES, 0.1 M
Hydrochloric acid, 0.1 M
Imidazole, 0.2 M
Magnesium chloride, 1 M
β-Mercaptoethanol, 1 M
MES, 0.1 M
Methanol, 10%
Modified Dulbecco's PBS
MOPS, 0.1 M
NAD, 2 mM
Nonidet P-40 (NP-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%
Tributylphosphine (TBP), 5 mM
TBS, 0.5x (12.5 mM Tris, 75 mM NaCl, pH 7.6)
TCEP, 20 mM
Thiourea, 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, 0.25x (6.25 mM Tris, 48 mM glycine, 0.025% SDS)
Triton X-100, 0.05%
Tween 20, 0.01%
Urea, 4 M

Appendix B

Reagents Compatible With the Bio-Rad Protein Assay When Using the Standard Procedure

Acetate, 0.6 M
Acetone
Acid pH
Adenosine, 1 mM
Amino acids
Ammonium sulfate, 1 M
Ampholytes, pH 3–10, 0.5%
ATP, 1 mM
Barbital
BES, 2.5 M
Boric acid
Cacodylate-Tris, 0.1 M
CDTA, 50 mM
Citrate, 50 mM
Deoxycholate, 0.1%
DNA, 1 mg/ml
DTT, 1 M
Eagle's MEM
Earle's salt solution
EDTA, 0.1 M
EGTA, 50 mM
Ethanol
Formic acid, 1 M
Fructose
Glucose
Glutathione
Glycerol, 99%
Glycine, 0.1 M
Guanidine-HCl
Hank's salt solution
HEPES, 0.1 M
Magnesium chloride, 1 M
Malic acid, 0.2 M
β-Mercaptoethanol, 1 M
MES, 0.7 M
Methanol
MOPS, 0.2 M
NAD, 1 mM
Peptone
Phenol, 5%
Phosphate, 1 M
PIPES, 0.5 M
Polyadenylic acid, 1 mM
Polypeptides (MW <3,000)
Potassium chloride, 1 M
Pyrophosphate, 0.2 M
rRNA, 0.25 mg/ml
tRNA, 0.4 mg/ml
Total RNA, 0.3 mg/ml
SDS, 0.1%
Sodium chloride, 5 M
Sodium phosphate
Sodium thiocyanate, 3 M
Streptomycin sulfate, 20%
Thymidine, 1 mM
Tricine
Tris, 2 M
Triton X-100, 0.1%
Tyrosine, 1 mM
Urea, 6 M
Vitamins

Appendix C

Reagents Compatible With the *DC* Protein Assay When Using the Standard Procedure

Ammonium sulfate, 0.5 M
Brij 35, 1%
$CaCl_2$, 50 mM
$C_{12}E_8$,* 0.2%
CHAPS, 1%
CHAPSO, 1%
DTT, 1 mM
EDTA, 25 mM
Guanidine-HCl, 0.4 M
Hydrochloric acid, 0.5 M
Nonidet P-40 (NP-40), 2%
Octyl-β-glucoside, 1%
SDS, 10%
Sodium azide, 0.05%
Sodium hydroxide, 0.5 M
Thesit, 1%
Tris, pH 8, 0.1 M
Triton X-100, 1%
Tween 20, 1%
Urea, 4 M

* Octaethyleneglycol dodecyl ether

Appendix D

Reagents Compatible With the *RC DC* Protein Assay When Using the Standard Procedure

CHAPS, 2%
DTT, 350 mM
EDTA, 0.1 M
Imidazole, 0.5 M
Laemmli buffer (with 5% β-mercaptoethanol)
β-Mercaptoethanol, 10%
ReadyPrep sequential extraction reagent 2**
ReadyPrep sequential extraction reagent 3***
Sodium hydroxide, 2.5 mM
Tributylphosphine (TBP), 2 mM
Tween 20, 2%
Triton X-100, 2%
Tris, pH 8.4, 0.5 M

** Catalog #163-2103: 40 mM Tris, 8 M urea, 4% (w/v) CHAPS, 0.2% (w/v) Bio-Lyte® 3/10 ampholyte, 2 mM TBP

***Catalog #163-2104: 40 mM Tris, 5 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (w/v) SB 3-10, 0.2% (w/v) Bio-Lyte 3/10 ampholyte, 2 mM TBP

Interference may be caused by chemical-protein and/or chemical-dye interactions. These appendices list known chemical reagents that do not directly affect the development of dye color. Since every protein-chemical reagent combination has not been assayed, it is possible that some of the listed reagents produce interference in combination with certain proteins. However, with respect to bovine serum albumin and γ-globulin, the listed reagents show little or no interference.

The concentrations of reagents are for the standard assay procedure. Equivalent concentrations of reagents compatible with the microassay procedure are 1/40 of those listed for the standard assay procedure. This is due to the difference in sample-to-dye ratio between the standard and microassay procedures.

Ordering Information

Catalog #	Description
500-0201	Quick Start Bradford Protein Assay Kit 1, includes 1 L 1x dye reagent, BSA standard (5 x 2 mg/ml)
500-0202	Quick Start Bradford Protein Assay Kit 2, includes 1 L 1x dye reagent, BSA standard set (2 sets of 7 standards, 0.125–2.0 mg/ml)
500-0203	Quick Start Bradford Protein Assay Kit 3, includes 1 L 1x dye reagent, bovine γ-globulin standard (5 x 2 mg/ml)
500-0204	Quick Start Bradford Protein Assay Kit 4, includes 1 L 1x dye reagent, bovine γ-globulin standard set (2 sets of 7 standards, 0.125–2.0 mg/ml)
500-0205	Quick Start 1x Dye Reagent, 1 L
500-0206	Quick Start Bovine Serum Albumin Standard, 5 x 2 mg/ml
500-0207	Quick Start Bovine Serum Albumin Standard Set, 2 sets of 7 standards, 0.125–2.0 mg/ml
500-0208	Quick Start Bovine γ-Globulin Standard, 5 x 2 mg/ml
500-0209	Quick Start Bovine γ-Globulin Standard Set, 2 sets of 7 standards, 0.125–2.0 mg/ml
500-0006	Bio-Rad Protein Assay Dye Reagent 5x Concentrate, based on method of Bradford, 450 ml supplied without a standard
500-0001	Bio-Rad Protein Assay Kit I, includes 450 ml dye reagent concentrate, bovine γ-globulin standard
500-0002	Bio-Rad Protein Assay Kit II, includes 450 ml dye reagent concentrate, bovine serum albumin standard
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
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
500-0113	*DC* Protein Assay Reagent A, alkaline copper tartrate solution, 250 ml
500-0114	*DC* Protein Assay Reagent B, dilute Folin reagent, 1 L
500-0115	*DC* Protein Assay Reagent S, surfactant solution, 5 ml
500-0116	*DC* Protein Assay Reagents Package, includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml of surfactant solution
500-0121	*RC DC* Protein Assay Kit I, includes *RC* reagents package, *DC* protein assay reagents package, bovine γ-globulin standard, 500 standard assays
500-0122	*RC DC* Protein Assay Kit II, includes *RC* reagents package, *DC* protein assay reagents package, bovine serum albumin standard, 500 standard assays
500-0120	*RC DC* Protein Assay Reagents Package, includes *RC* reagents package, *DC* protein assay reagents package, 500 standard assays
500-0119	*RC* Reagents Package, contains *RC* reagent I (250 ml), *RC* reagent II (250 ml), 500 standard assays
500-0117	*RC* Reagent I, 250 ml
500-0118	*RC* Reagent II, 250 ml
500-0005	Protein Standard I, bovine γ-globulin, reconstituted volume 20 ml
500-0007	Protein Standard II, bovine serum albumin, reconstituted volume 20 ml
170-2501	SmartSpec™ 3000 Spectrophotometer
Related Products	
223-9950	Disposable Polystyrene Cuvettes, 3.5 ml, 100
223-9955	Semimicro Disposable Polystyrene Cuvettes, 1.5 ml, 100
224-0096	Costar 96-Well Flat-Bottom EIA Plates, polystyrene, 5 per package, box of 100
224-4888	8-Pette Adjustable-Volume 8-Channel Pipet, 20–200 µl
224-4880	12-Pette Adjustable-Volume 12-Channel Pipet, 20–200 µl
170-6850	Benchmark™ Microplate Reader
170-6750	Model 550 Microplate Reader
170-7009	Model 1575 Immunowash Microplate Washer

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Nonidet is a trademark of Shell International Petroleum Co. Thesit is a trademark of Desitin Arzneimittel GmbH.
Triton is a trademark of Rohm and Haas.

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