



PROTEIN ASSAYS

Bio-Rad Protein Assay

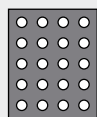
Quick Guide

Standard Assay



200–1,500 $\mu\text{g/ml}$

Microplate Standard Assay



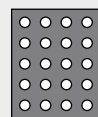
50–500 $\mu\text{g/ml}$

Micro Assay



1.2–25 $\mu\text{g/ml}$

Microplate Microassay



8–80 $\mu\text{g/ml}$

Prepare 1 part dye reagent with 4 parts DI H₂O

Pipet standards or samples into test tubes or microplate

100 μl

10 μl

800 μl

160 μl

Add dye reagent

5 ml

200 μl

200 μl

40 μl

Mix

Incubate for 5 to 60 min at room temperature

Measure absorbance at 595 nm; color will be stable for 1 hr

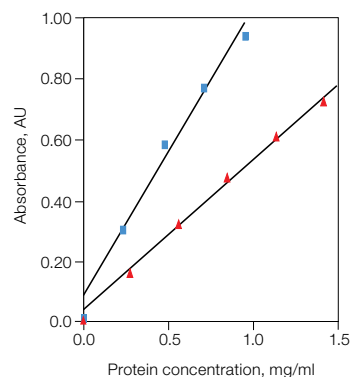
Bradford-based*; includes standards and prediluted reagent

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

Setting Up a Standard Curve

Determine protein concentration by plotting the absorbance vs. concentration of known standards. Use the resulting curve to determine the concentration of unknown proteins based on their absorbance.

Note: The best standard to use is a purified sample of your target protein. If this is not available, use Bio-Rad's bovine serum albumin or bovine γ -globulin to make your standard curve.



Standard curve generation using known standards.

A typical standard curve for Bradford-based assays, including Bio-Rad Protein Assays. Bovine serum albumin (\blacksquare); bovine γ -globulin (\blacktriangle).

For Best Results

- Run a standard curve with each protein assay
- Run at least 3 replicates of all standards and samples
- Process the sample and standards the same way to ensure that differences in color intensity are due only to differences in protein concentration
- Make sure the sample and standard fall within the same concentration range

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Reagent Compatibility

The following reagents were tested for their compatibility with the protein assay. Concentrations represent maximum concentrations for standard assay. ✓ = compatible.

Reagent	Concentration
Acetate	0.6 M
Acetone	✓
Acid pH	✓
Adenosine	0.001 M
Ammonium sulfate	1 M
Ampholytes pH 3–10	0.5%
ATP	0.001 M
Barbital	✓
BES	2.5 M
Boric acid	✓
Cacodylate-Tris	0.1 M
CDTA	0.05 M
Citrate	0.05 M
Deoxycholate	0.1%
Dithiothreitol (DTT)	1 M
DNA	1 mg/ml
Eagle's MEM	✓
Earle's salt solution	✓
EDTA	0.1 M
EGTA	0.05 M
Ethanol	✓
Formic acid	1.0 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
2-Mercaptoethanol	1 M
MES	0.7 M
Methanol	✓
MOPS	0.2 M
NAD	0.001 M

Reagent	Concentration
NaSCN	3 M
Peptone	✓
Phenol	5%
Phosphate	1 M
PIPES	0.5 M
Polyadenylic acid	0.001 M
Polypeptides (MW<3000)	✓
Potassium chloride	1 M
Pyrophosphate	0.2 M
rRNA	0.25 mg/ml
SDS	0.1%
Sodium chloride	5 M
Sodium phosphate	✓
Streptomycin sulfate	20%
Thymidine	0.001 M
Total RNA	0.30 mg/ml
Tricine	✓
Tris, pH 8	2 M
Triton X-100	0.1%
tRNA	0.4 mg/ml
Tyrosine	0.001 M
Urea	6 M
Vitamins	✓

Ordering Information

Catalog #	Description
5000001	Bio-Rad Protein Assay Kit I , includes dye concentrate and bovine γ -globulin standard
5000002	Bio-Rad Protein Assay Kit II , includes dye concentrate and bovine serum albumin standard

Microplate Reader

1681130	iMark™ Microplate Absorbance Reader
2240096	Costar 96-Well Flat-Bottom EIA Plate , 100 plates

Spectrophotometer

1702525	SmartSpec™ Plus Spectrophotometer
1702510	trUView™ Cuvettes , pkg of 50
1702511	trUView Cuvettes , pkg of 100
2239950	Standard Disposable Polystyrene Cuvettes , 3.5 ml, pkg of 100
2239955	Semimicrovolume Disposable Polystyrene Cuvettes , 1.5 ml, pkg of 100
1702502	Standard Cuvette , 1–3.5 ml, quartz
1702503	Semimicrovolume Cuvette , 0.5–1.4 ml, quartz
1702504	Microvolume Cuvette , 200–700 μ l, quartz
1702505	Submicrovolume Cuvette , 80–150 μ l, quartz

Visit bio-rad.com/proteinassaykits for more information.

Costar is a trademark of Corning Incorporated. Triton is a trademark of Dow Chemical Company.



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Web site bio-rad.com USA 1 800 424 6723 Australia 61 2 9914 2800 Austria 43 1 877 89 01 177 Belgium 32 (0)3 710 53 00 Brazil 55 11 3065 7550 Canada 1 905 364 3435 China 86 21 6169 8500 Czech Republic 420 241 430 532 Denmark 45 44 52 10 00 Finland 358 09 804 22 00 France 33 01 47 95 69 65 Germany 49 89 31 884 0 Hong Kong 852 2789 3300 Hungary 36 1 459 6100 India 91 124 4029300 Israel 972 03 963 6050 Italy 39 02 216091 Japan 81 3 6361 7000 Korea 82 2 3473 4460 Mexico 52 555 488 7670 The Netherlands 31 (0)318 540 666 New Zealand 64 9 415 2280 Norway 47 23 38 41 30 Poland 48 22 331 99 99 Portugal 351 21 472 7700 Russia 7 495 721 14 04 Singapore 65 6415 3188 South Africa 27 (0) 861 246 723 Spain 34 91 590 5200 Sweden 46 08 555 12700 Switzerland 41 026674 55 05 Taiwan 886 2 2578 7189 Thailand 66 662 651 8311 United Arab Emirates 971 4 8187300 United Kingdom 44 020 8328 2000

