

Bradford Reagent, ready-to-use #R1271

TABLE OF CONTENTS

TABLE OF CONTENTS.....	1
STORAGE	2
DESCRIPTION	2
ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED	2
IMPORTANT NOTES.....	3
A. Standard assay in test tubes	4
B. Standard assay in microplates	5
C. Micro assay in test tubes.....	6
D. Micro assay in microplates	7
ADDITIONAL INFORMATION	8
Interfering substances	8
Response characteristics for different proteins	11
Reference.....	11
RELATED PRODUCTS	12
QUALITY CONTROL	12
SAFETY INFORMATION	13

Bradford Reagent, ready-to-use #R1271, 1 liter

for 1000 standard test tube assays or 4000 standard microplate assays.

STORAGE

Bradford reagent, ready-to use, should be stored at 4 °C and protected from light.

DESCRIPTION

Bradford reagent, ready-to-use is designed for rapid and accurate estimation of total protein concentration in solution.

The Bradford protein assay relies on the formation of complexes between Coomassie Brilliant Blue G-250 dye and proteins in solution. This binding causes a shift in the absorption maximum of the dye from 465 nm to 595 nm (1). The concentration of the protein sample is determined by referencing the absorption to a series of standard protein dilutions assayed in parallel.

The quantification procedure is simple and rapid. The protein sample is mixed with the reagent at room temperature, the absorbance of the solution is measured at 595 nm. The absorption at 595 nm is proportional to the amount of protein present in the solution. The dye binding process is complete in approximately 5 min with color stability for 1 hour.

Sufficient reagent is supplied for 1000 standard test tube assays or 4000 standard microplate assays. The working range of the Bradford reagent is 1-2000 µg/ml of protein.

For your convenience use pre-diluted protein standards for the creation of standard curve.

Fermentas offers two sets of pre-diluted protein standards as separate products:

Bovine Serum Albumin Standard Set, ready-to-use (#R1281),

Bovine Gamma Globulin Standard Set, ready-to-use (#R1291).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

For assay in cuvettes

- Spectrophotometer capable of measuring absorbance at 595 nm.
- Vortex mixer.
- Test tubes.
- Quartz, optical glass or disposable cuvettes.
- Protein standard solutions.

For assay in 96-well microplates

- Microplate reader capable of measuring absorbance at 595 nm.
- Microplate mixer (or multichannel pipette).
- Microplates.
- Protein standard solutions.

IMPORTANT NOTES

General considerations

- There are four protocols for use of the Bradford reagent, ready-to-use. Depending on the protein concentration, standard or micro assay can be used. Depending on the volume of protein sample, the assay can be performed in test tubes or microplates.

Table 1. Assay formats.

Standard Assay		Micro Assay	
Working range: 100-2000 µg/ml Superior linear range: 125-1000 µg, BSA 125-1500 µg, BGG		Working range: 1-25 µg/ml Superior linear range: 1.25-10 µg, BSA 1.25-20 µg/ml, BGG	
Test Tube	Microplate	Test Tube	Microplate
20 µl sample 1 ml Bradford reagent, ready-to-use	5 µl sample 250 µl Bradford reagent, ready-to-use	750 µl sample 750 µl Bradford reagent, ready-to-use	120 µl sample 120 µl Bradford reagent, ready-to-use

BSA – bovine serum albumin.

BGG – bovine gamma globulin.

- Each assay should include three types of samples:
 - blank: contains reagent and dilution buffer without protein;
 - protein standard: contains reagent and protein of known concentration;
 - sample: contains reagent and protein of unknown concentration.
- Use the same solution for diluting both the protein standards and samples with unknown protein concentration.

Note. If the Bovine Serum Albumin Standard Set, ready-to-use (#R1281), or Bovine Gamma Globulin Standard Set, ready-to-use (#R1291) is used, 0.15 M NaCl, 0.05% NaN₃ solution should be used as diluent.
- Protein solutions are typically assayed in duplicate.
- Substances compatible with the Bradford reagent, ready-to-use, are listed in **Table 4** (p. 9).

Preparations before use

- Warm up the spectrophotometer or microplate reader before use.
- Gently mix the reagent solution by inverting the bottle a few times before each use.
- Aliquot the amount of the reagent needed and equilibrate to room temperature.

PROTOCOLS

A. Standard assay in test tubes

Step	Procedure
1	Gently mix the reagent solution by inverting the bottle a few times before each use, aliquot the amount of the reagent needed, and equilibrate it to room temperature.
2	Pipette 20 µl of each pre-diluted standard or sample to be assayed into appropriately labeled test tubes. Note. If pre-diluted standards are not available, standards of appropriate concentrations should be created by serial dilution of 2 mg/ml standard protein stock solution.
3	Add 1 ml of Bradford reagent, ready-to-use , to each tube and mix well.
4	Incubate at room temperature for 5 min . Note. Do not incubate longer than 60 min.
5	Transfer sample into cuvette.
6	Set the spectrophotometer to 595 nm . Zero the instrument with the blank sample. Measure the absorbance of standards and sample solutions.
7	Create a standard curve by plotting the absorbance at 595 nm vs. protein concentration of each protein standard (mg/ml).
8	Determine the protein concentration of unknown samples by comparing their absorbance values against the standard curve.

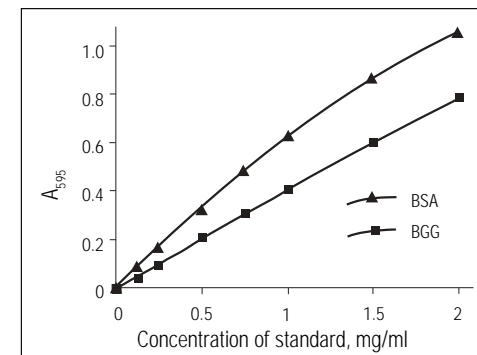


Fig. 1. Standard curve obtained using Bradford reagent, ready-to-use, in standard assay.

BSA – bovine serum albumin.

BGG – bovine gamma globulin.

B. Standard assay in microplates

Step	Procedure
1	Gently mix the reagent solution by inverting the bottle a few times before each use, aliquot the amount of the reagent needed, and equilibrate it to room temperature.
2	Pipette 5 µl of each pre-diluted standard or sample into individual microplate wells.
3	Add 250 µl of Bradford reagent, ready-to-use , to each well and mix using a microplate mixer. <i>Note. Alternatively, use a multichannel pipette for mixing.</i>
4	Incubate at room temperature for 5 min . <i>Note. Do not incubate longer than 60 min.</i>
5	Set the microplate reader to 595 nm . Zero the instrument with the blank sample. Measure the absorbance of standards and sample solutions.
6	Create a standard curve by plotting the absorbance at 595 nm vs. protein concentration of each standard (mg/ml).
7	Determine the protein concentration of unknown samples by comparing their absorbance values against the standard curve.

C. Micro assay in test tubes

Step	Procedure
1	Gently mix the reagent solution by inverting the bottle a few times before each use, aliquot the amount of the reagent needed, and equilibrate it to room temperature.
2	Pipette 750 µl of each pre-diluted standard (see Table 2) or sample to be assayed into appropriately labeled test tubes.
3	Add 750 µl of Bradford reagent, ready-to-use , to each tube and mix well.
4	Incubate at room temperature for 5 min . <i>Note. Do not incubate longer than 60 min.</i>
5	Transfer the samples into cuvettes.
6	Set the spectrophotometer to 595 nm . Zero the instrument with the blank sample. Measure the absorbance of standards and sample solutions.
7	Create a standard curve by plotting the absorbance at 595 nm vs. protein concentration of each standard (µg/ml).
8	Determine the protein concentration of unknown samples by comparing their absorbance values against the standard curve.

Table 2. Dilution chart for micro assay performed in test tube.

Tube No.	Protein standard		Volume of diluent, µl	Final concentration of standard, µg/ml
	Stock concentration, mg/ml	Required volume, µl		
1	2	25	1975	25
2	2	20	1980	20
3	1.5	20	1980	15
4	1	20	1980	10
5	0.5	20	1980	5
6	0.25	20	1980	2.5
7	0.125	20	1980	1.25
8 (blank)	-	-	2000	0

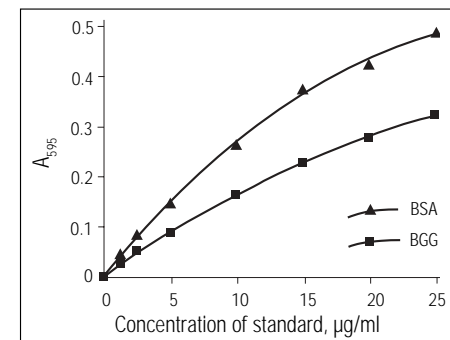


Fig. 2. Standard curve obtained using Bradford reagent, ready-to-use, in micro assay.

BSA – bovine serum albumin.
BGG – bovine gamma globulin.

D. Micro assay in microplates

Step	Procedure
1	Gently mix the reagent solution by inverting the bottle a few times before each use, aliquot the amount of the reagent needed, and equilibrate it to room temperature.
2	Pipette 120 µl of each pre-diluted standard (see Table 3) or sample to be assayed into individual microplate wells
3	Add 120 µl of Bradford reagent, ready-to-use , to each well, mix properly using a microplate mixer. Note. For mixing alternatively use a multichannel pipette.
4	Incubate at room temperature for 5 min. Note. Do not incubate longer than 60 min.
5	Set the microplate reader to 595 nm . Zero the instrument with the blank sample. Measure the absorbance of standards and sample solutions.
6	Create a standard curve by plotting the absorbance at 595 nm vs. protein concentration of each standard (µg/ml).
7	Determine the protein concentration of unknown samples by comparing their absorbance values against the standard curve.

Table 3. Dilution chart for micro assay performed in microplate.

Tube No.	Protein standard		Volume of diluent, µl	Final concentration of standard, µg/ml
	Stock concentration, mg/ml	Required volume, µl		
1	2	10	790	25
2	2	10	990	20
3	1.5	10	990	15
4	1	10	990	10
5	0.5	10	990	5
6	0.25	10	990	2.5
7	0.125	10	990	1.25
8 (blank)	-	-	1000	0

TROUBLESHOOTING

Problem	Cause & Solution
The protein standard and sample absorbance is lower than expected	Expired or improperly stored reagent. Store the reagent at 4°C. Replace reagent if it is past the expiration date.
	Reagent is cold. Warm the reagent to ambient temperature.
Protein sample absorbance is lower than expected	Suboptimal protein concentration. Follow the micro assay protocol.
	Samples may contain substances that interfere with the reaction. Check the compatibility chart for Bradford reagent, ready-to-use (Table 4). Dilute the sample. Dialyze the sample.
	Molecular weight of the protein is less than 3 kDa. The Bradford reagent, ready to use, is compatible with the majority of proteins >3 kDa. For proteins <3 kDa, other quantification assays such as BCA (bicinchoninic acid) could be more suitable.
Protein sample absorbance is higher than expected	Samples may contain substances that interfere with the reaction Check the compatibility chart for Bradford reagent, ready-to-use (Table 4). Dilute the sample. Dialyze the sample.
	Excessive protein concentration. Dilute the sample.
Precipitation occurs during the assay	Samples contain detergent. Dilute the sample.
	Bradford reagent is not properly mixed before use. Invert the bottle with reagent buffer several times to dissolve precipitates.
Need an absorbance at a wavelength other than 595 nm	595 nm filter is not available for spectrophotometer or plate reader. Measure the absorbance at any wavelength between 570-610 nm.

ADDITIONAL INFORMATION

Interfering substances

The Bradford assay is compatible with most commonly used biochemical reagents. However, certain detergents, flavonoids and basic buffers interfere with Coomassie-based protein assays by altering color development or causing reagent precipitation. Bradford reagent, ready-to-use, is compatible with low amounts of these chemicals. Information on most common substances and the maximum concentrations that can be present in the protein sample are given in the compatibility chart. The data is provided for the standard assay procedure. Concentrations compatible with the micro assay procedure are 1/25 of those listed in the table. Substances were considered compatible if the error rate in protein concentration estimation caused by the presence of the substance was less than or equal to 10%.

Table 4. Compatibility chart for Bradford reagent, ready-to-use.

Substance	Compatible Concentration
Detergents	
Tween 20	0.06%
Nonidet P-40 (NP- 40)	0.5%
CHAPS	5%
Triton X-100	0.1%
Triton X-114	0.1%
Brij® 58P	0.01%
Big CHAP	5%
Elugent® detergent	0.1%
SDS	0.02%
ASB-C7BzO	0.25%
Chelating agents	
EDTA	0.1 M
EGTA	0.1 M
Sodium citrate	0.05 M
Reducing and thiol-containing agents	
Potassium thiocyanate	1 M
DTT	10 mM
DTE	10 mM
2-Merkaptoethanol	1 M
Glucose	0.5 M
Glutathione	10 mM
TCEP-HCl	30 mM
Thiourea	1 M
Other reagents and solvents	
Ethanol	30%
Methanol	12%
Acetone	15%
Acetonitrile	12%
DMF	10%
DMSO	10%
Glycerol	50%
Urea	5 M
PMSF	1 mM
Avidin	2 ng/ul
d-Desthiobiotin	2.5 mM
Polyvinylpyrrolidone	10%
2-Pyrrolidinone	0.8 M
NDSB-201	1 M
Sodium deoxycholate	0.1%
Total RNA	0.3 mg/ml
DNA	1 mg/ml
Ampholyte 3/10	0.5%
Cell Lysis Buffer (#K0311)	undiluted
Nuclei Lysis Buffer (#K0311)	undiluted

Substance	Compatible Concentration
Other reagents and solvents	
ProteoJET™ Mammalian Cell Lysis Reagent (#K0301)	undiluted
Sodium hydroxide	100 mM
Hydrochloric acid	100 mM
Hydroxylamine	2 M
TFA (trifluoroacetic acid)	0.1%
Sucrose	0.3 M
Sodium orthovanadate in PBS, pH 7.2	1 mM
Poly(ethyleneimine) solution	1%
NaF (sodium fluoride)	5 mM
L-arginine monohydrochloride	1 M
L-lysine monohydrochloride	1 M
Phenol	0.1%
Imidazole	0.2 M
Maleic acid	0.1 M
TMANO (trimethylamine N-oxide)	0.8 M
TMAC (tetramethyl ammonium chloride)	0.8 M
PEG 4000	10%
PEG 6000	10%
Trehalose	1 M
Salts/Buffers	
Ammonium sulfate	1 M
Sodium phosphate	0.5 M
HEPES	0.2 M
Sodium acetate, pH 4.8	0.18 M
Potassium phosphate	1 M
MES, pH 6.1	0.1 M
Guanidine-HCl	1 M
Magnesium chloride	0.5 M
MOPS, pH 7.2	0.2 M
Potassium chloride	2 M
Sodium chloride	2 M
Tris base	1 M
Sodium bicarbonate	0.2 M
Sodium carbonate	0.1 M
ATP	2 mM
Sodium azide	0.5%
PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na ₂ HPO ₄ , 2 mM KH ₂ PO ₄ , pH 7.4)	undiluted
Zinc chloride in TBS, pH 7.2	10 mM
Calcium chloride in TBS, pH 7.2	10 mM
Boric acid	0.2 M
Cacodylate-Tris	0.1 M
Glycine	0.2 M
Tricine, pH 8.0	0.1 M
25 mM Tris, 192 mM glycine, pH 8.0	undiluted
25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.0	10X dilution

Response characteristics for different proteins

Quantification accuracy with the Bradford reagent is protein-dependent. The response of an individual protein depends on the: amino acid sequence, isoelectric point, structure, prosthetic groups, etc. Therefore for precise quantification use the same standard protein to create the calibration curve.

When relative protein concentration values are needed, any purified protein with a known concentration can be selected as a standard. The two most common protein standards for protein assays are BSA and BGG.

Table 5 shows protein-to-protein variations in color response. All proteins were tested in standard test tube protocol using 1 mg/ml protein concentration. The average response for BSA is normalized to 1.00 and average responses of the other proteins are expressed as a ratio to the response of BSA.

Table 5. Protein-to-protein variation using standard test tube protocol in the Bradford assay, using Bradford reagent, ready-to-use.

Protein tested	Ratio
Bovine serum albumin (BSA)	1.00
Bovine gamma-globulin (BGG)	0.65
Ovalbumin	0.56
β-lactoglobulin	0.64
Carbonic anhydrase	1.34
Lactatdehydrogenase	1.26
Lysozyme	0.66
β-galactosidase	0.65
REase Bsp98I	0.80
Chymotrypsinogen A	0.67
Aldolase (rabbit muscle)	0.63
Average ratio	0.82
Standard deviation	0.28
Coefficient of variation	33.65%

$$\text{Ratio} = \frac{\text{Absorbance of protein tested}}{\text{Absorbance of BSA}}$$

Reference

1. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.*, 72, 248-54, 1976.

RELATED PRODUCTS

Product	Amount	Catalog #
Bovine Serum Albumin Standard Set, ready-to-use	7 x 4 ml	R1281
Bovine Gamma Globulin Standard Set, ready-to-use	7 x 4 ml	R1291
ProteoJET™ Mammalian Cell Lysis Reagent	250 ml	K0301
ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit	50 preps	K0311
Spectra™ Multicolor Broad Range Protein Ladder	2 x 250 µl	SM1841
PageRuler™ Unstained Protein Ladder	2 x 250 µl	SM0661
PageRuler™ Prestained Protein Ladder	2 x 250 µl	SM0671
PageRuler™ Plus Prestained Protein Ladder	2 x 250 µl	SM1811
Unstained Protein Molecular Weight Marker	2 x 1000 µl	SM0431
Prestained Protein Molecular Weight Marker	2 x 250 µl	SM0441
PageBlue™ Protein Staining Solution	1 liter	R0571
PageSilver™ Silver Staining Kit	25 preps	K0681
10X Tris-glycine-SDS Electrophoresis Buffer	1 liter	B46
10X Tris-glycine Electrophoresis Buffer	1 liter	B47
10X Tris-tricine-SDS Electrophoresis Buffer	1 liter	B48
DualColor™ Protein Loading Buffer Pack	1000 samples	R1011
Protein Loading Buffer Pack	2000 samples	R0891

QUALITY CONTROL

Bradford reagent, ready-to-use is tested using Bovine Serum Albumin standard set, ready-to-use, in standard test tube assay.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

(1) Revised 12.12.2007

SAFETY INFORMATION

Bradford Reagent



C Corrosive

Risk phrases

R34 Causes burns.

Safety phrases

S20 When using do not eat or drink.

S23 Do not breathe gas/fumes/vapour/spray.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S60 This material and its container must be disposed of as hazardous waste.