LAMDA BIOTECH

PRODUCT INFORMATION

Catalog No.: G1001

Product Name: **Bradford Protein Assay**

Size: 1 Kit

Description: **Bradford Protein Assay** is a fast and easy protein assay based on Coomassie Blue G. It is compatible with many of the salts, solvents, buffers, reducing chemicals and chelating agents often used in protein samples. Assays can be performed in test tubes, microcentrifuge tubes and multi-well plate formats.

The Bradford Protein Assay is based on the equilibrium between three forms of Coomassie Blue G dye. Under acidic conditions, proteins bind to the reagent, causing a spectral shift from reddish/brown to blue.

Protein + **Coomassie Blue G** => **Red** (465nm) <=> **Green** (650nm) <=> **Blue-Protein** (595nm)

Absorption measured by a spectrophotometer at 595nm is proportional to the protein present in the solution. Using Bovine Serum Albumin (BSA) as a standard protein, the Standard Bradford Protein Assay is linear in concentrations between 50 and 1,500µg/ml, while the Low Protein Bradford Assay is linear from 1-50µg/ml.

While the Bradford Reagent is compatible with reducing agents frequently used to stabilize proteins in solution, it is only compatible with low concentrations of detergent. If the protein samples to be assayed have detergent(s) in the buffer, use **BCA Protein Assay** (Cat. No. G1002) or **Low Protein BCA Assay** (Cat. No. G1003).

Kit Contents*:

5X Bradford Assay Reagent:	150ml
Protein Standard Solution:	8ml

Precautions: This product is for research use only; not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage: Store all contents at 4°C.

Related products	Catalog No.
BCA Protein Assay	G1002
Low Protein BCA Assay	G1003

* Contents sufficient for 500 Standard Assays, 1,000 Low Protein Assays and up to 5,000 Microplate Assays.

G1001
Bradford Protein Assay

Protocols and Technical Instructions

Standard Protocol:

1. Prepare **Working Reagent** by mixing 1 volume of **5X Bradford Assay Reagent** with 4 volumes of H_2O.
2. Prepare all Standard Curve dilutions in the same buffer as the samples to be assayed. The **Standard Bradford Protein Assay** should yield a linear curve between 50 and 1500µg/ml.

Suggested Dilutions for Standard Assay:

Volume of Standard Curve Dilutions (**Protein Standard** = 2.0 mg/ml)		Diluent Volume	Final BSA Concentration
Blank:	dilution buffer only	1,000 µl	0
A:	750 µl **Protein Standard**	250 µl	1,500 µg/ml
B:	500 µl **Protein Standard**	500 µl	1,000 µg/ml
C:	500 µl dilution **A**	500 µl	750 µg/ml
D:	500 µl dilution **B**	500 µl	500 µg/ml
E:	500 µl dilution **D**	500 µl	250 µg/ml
F:	500 µl dilution **E**	500 µl	125 µg/ml
G:	400 µl dilution **F**	600 µl	50 µg/ml

3. Samples with unknown protein concentrations should be approximately 0.05-1.5 mg/ml of protein. If it is necessary to dilute these Unknowns, be sure to use the same buffer as with the Standard Curve dilutions.
4. Add 30µl of the Blank, each Standard Curve dilution and all Unknowns to separate test tubes.
5. Add 1.5ml **Working Reagent** to each tube in Step 4.
6. Mix the contents of the tubes gently.
7. In a spectrophotometer, measure the absorbance of the Standard Curve dilutions and all Unknowns at 595nm. Use the Blank reading as your baseline. ***Note:*** The protein-dye complex is stable for up to 60 minutes.
8. Plot net A_{595} *vs.* the protein concentrations in the Standard Curve dilutions on a graph.
9. Determine protein concentration in the Unknowns from the graph of the Standard Curve.

Microplate Protocol:

1. Prepare **Working Reagent** by mixing 1 volume of **5X Bradford Assay Reagent** with 4 volumes of H_2O.
2. Prepare Standard Curve dilutions as in the Standard Protocol.
3. Samples with unknown protein concentrations should be approximately 0.05-1.5 mg/ ml of protein. If it is necessary to dilute these Unknowns, be sure to use the same buffer as with the Standard Curve dilutions.
4. Pipette 5µl of the Blank, each Standard Curve dilution, and all Unknowns into separate wells in a 96 well plate.
5. Add 200µl **Working Reagent** to each well used, and mix on a shaker for approximately one minute.
6. Incubate the plate at room temperature for 5 to 45 minutes, then measure the absorbance at 595nm. ***Note:*** The protein-dye complex is stable for up to 60 minutes.
7. Subtract the Blank reading from the readings of each Standard Curve dilution, and all Unknowns to get net A_{595}.
8. Plot net A_{595} *vs.* the protein concentrations in the Standard Curve dilutions on a graph.
9. Determine protein concentration in the Unknowns from the graph of the Standard Curve.

G1001
Bradford Protein Assay

Low Protein Protocol:

1. Prepare 2 ml of each Standard Curve dilution in the same buffer as the samples to be assayed. The **Low Protein Bradford Assay** should yield a linear curve between 1 and 50 µg/ml.

Suggested Dilutions for Low Protein Assay:

	Volume of Standard Curve Dilutions (**Protein Standard** = 2.0 mg/ml)	Diluent Volume	Final BSA Concentration
Blank:	dilution buffer only	2,000 µl	0
A:	50 µl **Protein Standard**	1,950 µl	50 µg/ml
B:	40 µl **Protein Standard**	1,960 µl	40 µg/ml
C:	1,000 µl dilution **A**	1,000 µl	25 µg/ml
D:	1,000 µl dilution **B**	1,000 µl	20 µg/ml
E:	1,000 µl dilution **D**	1,000 µl	10 µg/ml
F:	1,000 µl dilution **E**	1,000 µl	5 µg/ml
G:	200 µl dilution **F**	800 µl	1 µg/ml

2. Samples with unknown protein concentrations should be approximately 1-50µg/ml of protein. If it is necessary to dilute these Unknowns, be sure to use the same buffer as with the Standard Curve dilutions.
3. Prepare a microcentrifuge tube for the Blank, each Standard Curve dilution, and Unknown sample(s).
4. Add 200µl of **5X Bradford Assay Reagent** to each microcentrifuge tube. Do ***not*** dilute the **5X Bradford Assay Reagent** before use in the Low Protein Protocol.
5. Add 800µl of each Standard Curve dilution and all Unknown samples to the above tubes and mix.
6. In a spectrophotometer, measure the absorbance of the Standard Curve dilutions and all Unknowns at 595nm. Use the Blank reading as your baseline. ***Note:*** The protein-dye complex is stable for up to 60 minutes.
7. Plot net A_{595} *vs.* the protein concentrations in the Standard Curve dilutions on a graph.
8. Determine protein concentration in the Unknowns from the graph of the Standard Curve.

Technical Tips

- High levels of detergent and reagents that change pH can interfere with the Bradford assay. Check for interference, and possibly correct for a small amount of it by diluting the Standard Curve in the same buffer as the Unknowns.
- The protein concentration in Unknown samples should be diluted to fall within the linear range of the assays.
- Be sure all glassware is either new or has been cleaned well.
- Common to all liquid Coomassie dye reagents, upon standing the dye will spontaneously form loose aggregates, which may become visible in as little as 60 minutes. Gentle mixing of the reagent by inverting the bottle will disperse the dye uniformly again. Similar aggregates will form after protein binding. These protein-dye aggregates can also be easily dispersed by mixing the reaction tube.

References:

1. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254. 1976.
2. Stoscheck, CM. Quantitation of Protein. *Methods in Enzymology* 182: 50-69 (1990).