

Bradford Reagent

<u>Product Name</u>	<u>Product Code</u>	<u>Kit Packing</u>
Bradford Reagent	ML106-100ML	100ml
	ML106-500ML	500ml

Introduction:

Bradford Reagent is formulated for rapid and accurate quantitative estimation of protein samples by following the Bradford assay method which was developed by Marion M. Bradford in 1976. This reagent is conveniently supplied as ready-to-use form and no dilution is required while performing the assay.

Description:

Bradford Reagent contains a dye, Coomassie brilliant Blue G-250, which has an absorbance maximum of 465 nm in unbound state. The Bradford protein assay is based upon the formation of complexes between Coomassie Brilliant Blue G-250 and the protein samples in solution. When the protein sample binds to the dye the color of the solution turns blue from brown and there is a shift in the absorption maximum of the dye from 465 nm to 595 nm. This dye binding procedure is completed within 5 minutes and the blue colored complex formed is stable for one hour. Thus, concentration of unknown protein sample can be derived by plotting its absorbance value on the standard curve. The standard curve is obtained from the absorbance readings of the series of standard protein dilutions assayed alongside the unknown sample.

Application:

HiMedia's Bradford Reagent provide accurate determination of protein concentration with most of the protein samples. Following are the features of Bradford Reagent.

1. Samples can be estimated visually or values can be determined with a standard Spectrophotometer or plate reader at 595nm
2. HiMedia's Bradford reagent is ready-to-use and no need to prepare working standards.
3. Bradford assay is much easier and faster than traditional Lowry method.
4. Detects protein concentration in the range 20 to 1500 µg/mL

Composition:

Bradford reagent consists of Coomassie Brilliant Blue G-250 in phosphoric acid and ethanol.

Properties:

Appearance : Brown colored solution
Clarity : Clear and free of particles
Suitability test : This solution has been tested and is suitable for use in quantitative estimation of protein samples by Bradford assay

Storage conditions:

Bradford Reagent has to be stored at 2 - 8 °C. Under recommended condition, the reagent is stable for 12 months.

Quality control:

Bradford Reagent is tested for quantitative estimation of unknown protein samples.

Estimation of unknown protein using standard curve (with BSA)

A. Test tube method:

1. Take seven test tubes and label them as Blank and 1 to 6.
2. Make dilutions of standard protein (BSA) with concentrations of 20, 16, 12, 8, 4 $\mu\text{g}/200 \mu\text{l}$ by transferring respective amount of BSA solution (stock: 1mg/ml) and adjusting it to a total volume of 200 μl by adding distilled water as mentioned in the following table.
3. Add 1ml of Bradford's Reagent to each test tube and mix the contents of each tube thoroughly by vortexing the tubes and incubate at room temperature (below 30°C) for 10 minutes.
4. Transfer the content of the tubes to cuvettes and measure the absorbance at 595 nm wavelength.

Tube No.	Blank	1	2	3	4	5	6
Conc. of BSA (μg)	0.0	4	8	12	16	20	Unknown sample
Amt of Stock (μl)	0.0	4	8	12	16	20	200 μl
Amt of diluent (μl)	200	196	192	188	184	180	
Amt of Bradford Reagent (ml)	1	1	1	1	1	1	1
Vortex the tubes and incubate at RT for 10 minutes							
Absorbance at 595 nm							

5. Plot a Standard Curve of absorbance at 595 nm on "Y" axis versus concentration of protein $\mu\text{g}/200 \mu\text{l}$ on "X" axis.
6. Record the value "x" of unknown sample from graph corresponding to the absorbance reading.

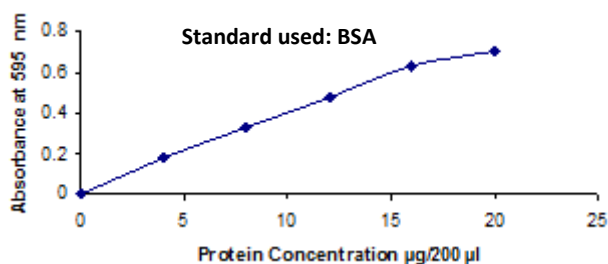


Fig: Standard curve for protein estimation by Bradford assay

Protein concentration can be calculated using following formula:

$$\text{Protein Concentration in Unknown Sample} = \frac{\text{Concentration of Unknown in "}\mu\text{g"}}{\text{Volume of sample in "}\mu\text{l"}}$$

$$= \text{_____ } \mu\text{g/ml}$$

B. 96-well plate assay:

This assay should be performed in a 96-well plate. It is very fast and easy to quantitate multiple protein samples at a time.

NOTE: For Standard Graph, 5 µl of 0.2-1.4 mg/ml of protein sample can be used.

Protocol:

1. Prepare protein standards by transferring respective amount of BSA solution (stock: 2 mg/ml) as mentioned in the following table.

Tube No.	Blank	1	2	3	4	5	6	7	8
Conc. of BSA (mg/ml)	0.0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	Unknown sample
Amt. of Stock (µl)	0.0	5	10	15	20	25	30	35	-
Amt. of diluent (µl)	50	45	40	35	30	25	20	15	
Amt. of Each standard and samples (µl)	5	5	5	5	5	5	5	5	5
Amt. of Bradford Reagent (µl)	250	250	250	250	250	250	250	250	250
Mix and incubate at RT for 5-10 minutes									
Absorbance at 595 nm									

2. Add 5µl of standard protein samples to each well of 96 plate.
3. Add 5 µl of unknown samples to the wells.

4. To each well, add 250µl of Bradford reagent.
5. Mix well by pipetting or keep it in a shaker for 15-30 seconds.
6. Incubate the samples for 5-10 minutes at room temperature and measure the absorbance at 595nm.
7. The absorbance readings must be taken within 30 minutes after the completion of incubation.
8. Note the corrected absorbance by subtracting the blank readings from the absorbance of standards and unknown samples.
9. Plot a Standard Curve of corrected absorbance at 595 nm on “Y” axis versus concentration of protein on “X” axis.
10. Record the value “x” of unknown sample from graph corresponding to the absorbance reading and determine the protein concentration.

Note: If the samples have been diluted, multiply the concentration of the sample by dilution factor.

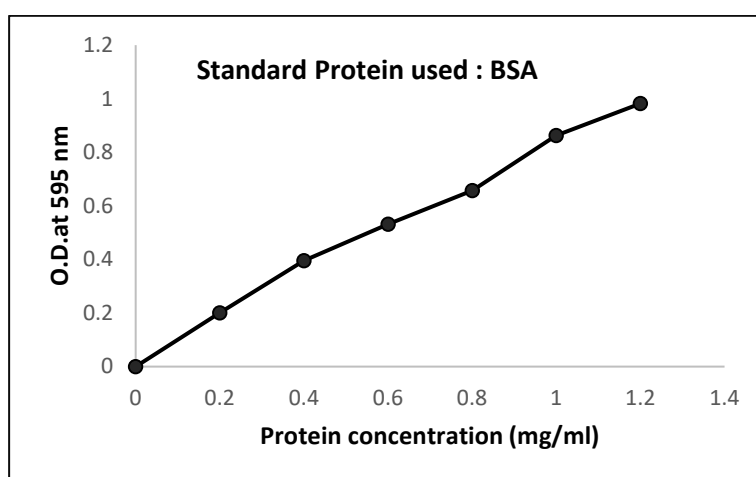


Fig 2: Standard curve for protein estimation by Bradford assay

Warning and Precautions

Not for Medicinal Use. Read the SDS carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

Performance of the solution is expected when the solution is stored at recommended temperature and within the expiry period.









Safety Information

The Bradford Reagent is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves and safety goggles when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbol

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIML106

Rev No.: 05

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Disclaimer :

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