5X Bradford Reagent for Protein Estimation

Product Name	Product Code	Kit Packing
5X Bradford Reagent For Protein Estimation	ML202 -100 ML	100ML

Introduction: HiMedia's Bradford Reagent for Protein Estimation is formulated for rapid and accurate quantitative estimation of protein samples by Bradford assay method which was developed by Marion M. Bradford in 1976. This reagent is conveniently supplied as a 5X concentrate solution which can be used as ready to use form as well as by diluting to 1X form.

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 5X Bradford reagent is ready-to-use as well as can be used by diluting 1 part of reagent concentrate with 4 parts distilled, deionized water.
3. Bradford assay is much easier and faster than traditional Lowry method.
4. Detects protein concentration in the range 1 to 1200 µg/mL

Kit Contents

Product Code	Reagents	Quantity	Storage
ML202	5X Bradford Reagent	100 ml	2 – 8°C

Materials required but not provided: Spectrophotometer, Test tubes, 1 ml cuvettes, 96-well plate, Micropipettes, Micropipette tips.

Storage conditions: Bradford Reagent should be stored at 2 - 8 °C

Tube Test 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 1, 5, 10, 15, 20, 25 µg/ml by transferring respective amount of BSA solution (stock: 1mg/ml) and adjusting it to a total volume of 1ml by adding distilled water as mentioned in the following table.
3. Pipette 800µl of Blank, each standard and unknown sample solutions into a clean, dry test tubes.

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4. Add 200 µl of 5X Bradford's Reagent concentrate 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 5- 10 minutes.
5. Transfer the content of the tubes to cuvettes and measure the absorbance at 595 nm wavelength.

Table no: 1

Tube No.	Blank	1	2	3	4	5	6	7
Conc. of BSA (µg/ml)	0.0	1	5	10	15	20	25	Unknown sample
Amt. of Stock (µl)	0.0	1	5	10	15	20	25	-
Amt. of diluent (µl)	1000	999	995	990	985	980	975	
Amt. of Each standard and samples (µl)	800	800	800	800	800	800	800	800
Amt. of 5X Bradford Reagent (µl)	200	200	200	200	200	200	200	200
	Vortex the tubes and incubate at RT for 5-10 minutes							
Absorbance at 595 nm								

5. Plot a Standard Curve of absorbance at 595 nm on "Y" axis versus concentration of protein µg/ml on "X" axis.
6. Record the value "x" of unknown sample from graph corresponding to the absorbance reading.

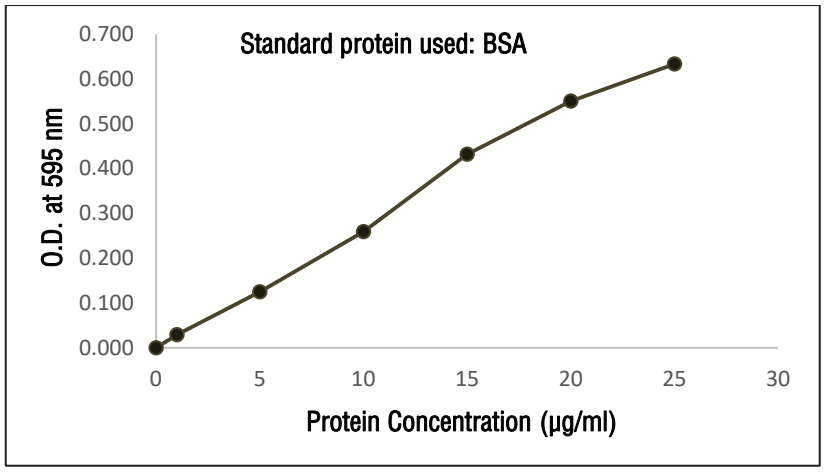


Fig 1: Standard curve for protein estimation by Bradford assay

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.

Standard Protocol for 96 well plate assay:

1. Prepare protein standards by transferring respective amount of BSA solution (stock: 2 mg/ml) as mentioned in the following table.
2. Add 5µl of standard protein samples to each well of 96-well plate.
3. Add 5 µl of unknown samples to the wells.
4. To each well, add 250µl of (1:4) diluted 5X 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 before 30 minutes or within 10 minutes after the completion of incubation.

Table no: 2

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

NOTE: For Standard Graph, 5 µl of 0.2-1.2mg/ml of protein sample can be used.

8. Plot a Standard Curve of absorbance at 595 nm on "Y" axis versus concentration of Protein on "X" axis.
9. Record the value "x" of unknown sample from graph corresponding to the absorbance reading and determine the protein concentration.

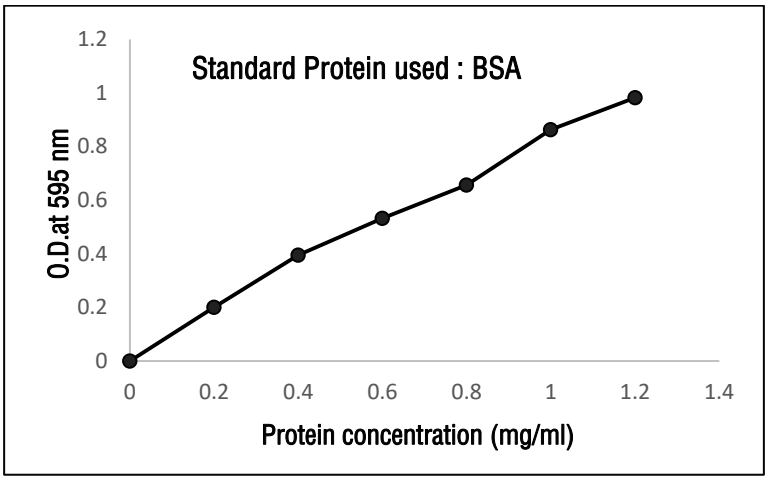


Fig 2: Standard curve for protein estimation by Bradford assay

Microassay procedure for 96 well plate:

1. Prepare protein standards by transferring respective amount of BSA solution (stock: 1 mg/ml) as mentioned in the following table.
2. Add 160 µl of standard protein samples, Blank, Unknown samples to each well of 96-well plate.
3. To each well, add 40µl of 5X Bradford reagent.
4. Mix well by pipetting or keep it in a shaker for 15-30 seconds.
5. Incubate the samples for 5-10 minutes at room temperature and measure the absorbance at 595nm.
6. The absorbance readings must be taken before 30 minutes or within 10 minutes after the completion of incubation.

Table no: 3

Tube No.	Blank	1	2	3	4	5	6	7
Conc. of BSA (µg/ml)	0.0	1	5	10	15	20	25	Unknown sample
Amt. of Stock (µl)	0.0	1	5	10	15	20	25	-
Amt. of diluent (µl)	1000	999	995	990	985	980	975	
Amt. of Each standard and samples (µl)	160	160	160	160	160	160	160	160
Amt. of 5X Bradford Reagent (µl)	40	40	40	40	40	40	40	40
	Mix and incubate at RT for 5-10 minutes							
Absorbance at 595 nm								

Note: For Standard Graph, 160 µl of 1-25 µg/ml of protein sample can be used.

7. Plot a Standard Curve of absorbance at 595 nm on "Y" axis versus concentration of Protein on "X" axis.
8. Record the value "x" of unknown sample from graph corresponding to the absorbance reading and determine the protein concentration.

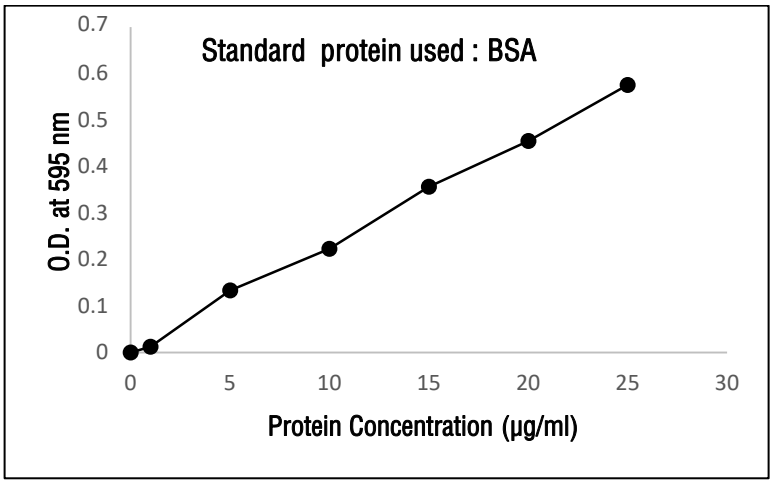
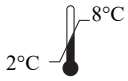


Fig 3: Standard curve for protein estimation by Bradford assay

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.



Storage temperature


Do not use if package is damaged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