Ver.250301

Bradford Kit for Protein Estimation

PC0010

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Introduction

Bradford Kit 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 kit contains ready-to-use Bradford Reagent and Protein Standard.

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

Bradford Reagent provides 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. 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
5. The Bradford kit is sufficient to perform at least 160 standard 3 ml assays

Kit Contents

Reagents	Quantity	Storage
Bradford Reagent	100mL	2 – 8^{o}C
Protein Standard (1mg/mL)	1mL	2 – 8^{o}C

Materials required but not provided

Spectrophotometer, Test tubes, 1mL disposable cuvettes, 96-well plate.

Storage conditions

Bradford Reagent and Protein Standard should be stored at 2-8°C. Under recommended condition, the kit is stable for 12 months.

Estimation of unknown protein using standard curve (with BSA)

1. Take seven test tubes and label them as Blank and 1 to 6.
2. Make dilutions of standard protein (BSA) with concentrations of 4, 8, 12, 16, 20µg/200µL by transferring respective amount of BSA solution (stock: 1mg/mL) and adjusting it to a total volume of 200µL 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^{o}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
Protein Standard (µL)	0.0	4	8	12	16	20	200µL
Water (µL)	200	196	192	188	184	180	
Bradford Reagent (mL)	1	1	1	1	1	1	1
Vortex the tubes and incubate at RT for 10 minutes							

5. Measure absorbance at 595nm
6. Plot a Standard Curve of absorbance at 595nm on "Y" axis versus concentration of protein µg/200µL on "X" axis.
7. Record the value "x" of unknown sample from graph corresponding to the absorbance reading.

Protein concentration can be calculated using following formula:

$$\text{Protein Concentration in Unknown Sample} = \frac{\text{Concentration of Unknown in "µg"}}{\text{Volume of sample in "µL"}} \times 5 = \_\ \mu g/mL$$

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 mg/mL of Protein Working Standard can be used.

Protocol

1. Prepare protein standards as mentioned above
2. Add 5µL of Protein Working Standard to each well of 96-well 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 5-45 minutes at room temperature and measure the absorbance at 595nm.
7. The absorbance readings must be taken before 60 minutes or within 10 minutes after the completion of incubation.
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.