Bradford Reagent

Product Overview

The Bradford Protein Assay is a fast, cost-effective, and straightforward method for determining protein concentration. This assay involves the reaction of a protein sample with the Bradford reagent, which contains Coomassie Brilliant Blue G-250 (CBBG) dye. The CBBG dye features two sulfonic acid groups and six phenyl groups that interact with proteins through positively charged and hydrophobic residues, respectively. When the dye binds to the protein, its absorbance shifts from 465 nm to 595 nm.

To perform the assay, the absorbance of various concentrations of a known protein standard is measured. These measurements are then used to create a standard curve by plotting absorbance against protein concentration and performing a linear regression analysis. The protein concentration of an unknown sample can be determined by measuring its absorbance and using the regression equation from the standard curve.

Bovine serum albumin (BSA) is the most commonly used standard protein because it is highly sensitive in this assay. Lysozyme is another option, as it represents a more typical protein in terms of hydrophobic content. However, since proteins can vary significantly in their amino acid compositions, the estimated protein concentration may differ. The most accurate standard for an assay would be the protein of interest if its concentration is known.

Catalog Details

RC33-1	100 mL
RC33-5	500 mL

Storage

- Store at 4°C.
- These products may be shipped on room temperature and should be stored at 4°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.

Protocol

Measuring Protein Standard

1. Warm up the spectrophotometer.

2. Pipet six different volumes [0 µl (0 µg), 10 µl (5 µg), 20 µl (10 µg), 30 µl (15 µg), 40 µl (20 µg), 50 µl (25 µg)] of 0.5 mg/ml BSA into separate cuvettes.

a. Other protein standards can be substituted for BSA. The tube with no protein standard in it serves as a blank.

3. Add 1.5 ml of Bradford Reagent to each tube.

4. Let the tubes incubate at room temperature for 2 to 3 min.

5. Measure the absorbance of each cuvette at 595 nm.

BSA/ Protein sample (0.5 mg/mL)	Nuclease free water	Bradford Reagent
Blank	50 µl	1.5 mL
10µl	40 µl	1.5 mL
20µl	30 µl	1.5 mL
30µl	20 µl	1.5 mL
40µl	10 µl	1.5 mL
50µl	-	1.5 mL

Standard Curve Generation

1. Make a scatter plot for the standard curve values. The X-axis will be µg of standard protein that were assayed; the Y-axis will be Abs 595nm that was measured.

2. Fit a linear trendline to the graph. Show the linear regression equation. Calculate the unknown amount of protein by using the linear regression equation, as shown in the calculations section of this document.

3. The unknown concentration of the original protein solution can be calculated by dividing the amount of protein by the volume of protein assayed and multiplying the quotient by the dilution factor, as shown in the calculations section.

4. Alternatively, a curvilinear (polynomial) trendline can be fitted to the graph. This polynomial regression equation may sometimes give a better estimation of protein concentration.

TIPS

- Always use a blank!
- Most detergents interfere with the Bradford Assay.
- Most reducing agents do not significantly interfere with the assay.
- Avoid quartz cuvettes because CBBG can bind to the quartz.

Other products/Services

cDNA Synthesis kits | SYBR GREEN Mastermix | DNA Markers | Enzymes | Extraction Kits

Gene Synthesis | CRISPR KO Vectors | PROBES for qPCR | sgRNA Synthesis | Protein Expression

Contact

info@dxbidt.com | +91-7349708807 | www.dxbidt.com