Bradford Protein Assay Kit

Product Description:

The detection principle of the Bradford method is that Coomassie Brilliant Blue G-250 binds to the basic and aromatic amino acids of the protein, especially arginine, in an acidic medium, at which point the solution turns blue, and the maximum absorption peak of the solution migrates from 465 nm to 595 nm. The protein concentration in the solution can be quantitatively determined by detecting the absorbance value at 595 nm, according to the change in the color of the solution is proportional to the protein concentration.

The Bradford Protein Assay Kit is developed according to the Bradford method and is one of the commonly used methods for protein quantification, which can achieve fast, stable, and highly sensitive protein concentration determination with good linearity of detection data.

The kit has the following advantages: extremely fast detection speed, compared with BCA, 10-20 samples can be completed in less than 10 minutes, greatly reducing the detection time, high detection sensitivity, 0.5 μg protein detection, good standard curve linearity, 5 μL volume of samples or standards have a good linear relationship in the concentration range of 0.1-1.5 mg/mL.

Composition and storage conditions

Components	K4103-500 T	K4103-1000 T
G250 reagent	125 mL	2 X 125 mL
Bovine serum albumin (BSA)	10 mg	20 mg
Bradford Protein Assay Reagent	2 mL	2 X 2 mL
Store the components at 4°C for a year.		

Experimental manipulation

1. Preparation of protein standards

a. Add 2 mL of Bradford Protein Assay Reagent to a tube of Bovine serum albumin (10 mg), dissolve thoroughly, and make a 5 mg/mL protein standard solution. It can be used immediately after formulation or can be stored at -20°C for long periods of time. *Note: Other protein standards are configured according to the final concentration of 5 mg/mL.*

b. Seven protein standards of 0, 0.125, 0.25, 0.5, 0.75, 1, and 1.5 mg/mL were prepared according to the table below. Take care to mix well each time you dilute it.

Numbering	Dilute the volume of liquid	Standard volume	Final concentration
A	70 μL	5 mg/mL BSA 30 μL	1.5 mg/mL
B	30 μL	Take 60 μL from tube A	1 mg/mL
C	20 μL	Take 60 μL from tube B	0.75 mg/mL
D	30 μL	Take 60 μL from tube C	0.5 mg/mL
E	60 μL	Take 60 μL from tube D	0.25 mg/mL
F	60 μL	Take 60 μL from the E tube	0.125 mg/mL
G	60 μL	0 μL	0 mg/mL

2. **Protein concentration determination**

a. 5 μL of different concentrations of protein standards were added to the protein standard wells of the 96-well plate.

b. Take 5 μL of the sample and place it into the sample well of the 96-well plate. If the sample is less than 5 μL, add the standard dilution to 5 μL and note the sample volume. Note: What solution is the protein sample in, and what solution should the standard be diluted. For simplicity, if the protein sample is in a solution that does not contain detergents, reducing agents, etc., the standard can also be diluted with 0.9% NaCl, PBS, or water.

c. 250 μL of G250 reagent was added to each of the above wells.

d. The absorbance value at A595 was measured by microplate reader, which could be measured immediately or within 2 h.

e. The protein concentration in the sample is calculated from the standard curve and the sample volume used.

Precautions

1. Make sure that the 5 μL volume of sample or standard is within the 0.1-1.5 mg/mL concentration range.
2. In contrast to the BCA and Lowry methods, the Bradford method determines protein concentrations that are not affected by the chemicals present in most samples, especially reducing reagents. Concentrations of β-mercaptoethano and dithiothreitol (DTT) can be as high as 1 M in the sample. However, this product is affected by a slightly higher concentration of detergent, and it is necessary to ensure that the SDS is less than 0.01%, Triton X-100 is less than 0.05%, and Tween 20, 60, 80 is less than 0.015%. Bradford Detergent Compatible or BCA Protein Concentration Assays are recommended for samples containing detergents.
3. Protein standards should be mixed first after all are dissolved, and then diluted into a series of protein standards of different concentrations.

4. Returning the G250 regent to room temperature before use is beneficial for improving detection sensitivity.
5. A microplate reader with a wavelength of 560-610 nm is selected, and the optimal detection wavelength is 595 nm, which can be used with a 96-well plate.
6. This product is for scientific research purposes only.

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