Detergent Compatible 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.

This kit is a detergent-compatible Bradford protein concentration assay kit, which is well compatible with common detergents such as 1% Triton X-100, 1% Tween 20, 1% SDS, 1% NP-40 and 1% Brij35, etc., and is compatible with high concentrations of reducing agents and faster detection than the BCA method.

This kit has the following advantages: the detection speed is extremely fast, compared with the BCA method, 10-20 samples can be completed in less than 10 minutes, which greatly shortens the detection time, the detection sensitivity is high, the detection of 0.5 μg protein can be realized, the linear relationship of the standard curve is good, it is compatible with common detergents, and for 5 μL volume of sample or standard, there is a good linear relationship over the concentration range of 0.1-1.5 mg/mL.

Composition and storage conditions

Components	K4104-800 T
G250 reagent (detergent compatible)	2 X 125 mL
Bovine serum albumin (BSA)	20 mg
Bradford Protein Assay Reagent	2 mL
Store the components at 4°C for a year.	

Experimental manipulation

1. Preparation of protein standards

   a. Add 1 mL of Bradford Protein Assay Reagent to a tube of Bovine serum albumin (20 mg), dissolve thoroughly, and make a 20 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 20 mg/mL.

b. Standard diluent selection: Method 1 - The standard is diluted with the solution components of the protein sample, and Method 2 - For simplicity, the standard can also be diluted with 0.9% NaCl, PBS, or water if the solution in which the protein sample is located does not contain detergent. Protein standard (20 mg/ml BSA) If cryopreserved, thaw completely and mix well before use.

c. 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. If necessary, the protein standard can be increased to 0.0625 mg/ml.

Numbering	Dilute the volume of liquid	Standard volume	Final concentration
A	92.5 μL	20 mg/mL BSA 7.5 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. 10 μL of protein standards of different concentrations were added to the protein standard wells of the 96-well plate.

b. 10 μL of sample was placed in the sample well of a 96-well plate. If the sample is less than 10 μL, add the standard dilution to 10 μL and note the sample volume.

c. 300 μL of G250 reagent (detergent compatible) 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 30 minutes. *[Note] For the case of some specific detergents, the test data will change to a certain extent within 2 h, but there will still be a good linear relationship.*

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

Notes

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 other methods for determining protein concentration, such as the BCA method and Lowry, the protein concentration determined with this kit is not affected by the chemicals present in most samples, especially reducing reagents and detergents. Concentrations of β-mercaptoethanol (β-Mercaptoethanol) and dithiothreitol (DTT) can be as high as 1 M in the sample. At the same time, the kit is also compatible with all of the common detergents mentioned above. The BCA method is resistant

to a wide range of common detergents, but not to high concentrations of reducing agents.

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 use only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com