5× Coomassie Brilliant Blue G-250(for protein quantification)

Item number: PC0015

Specification: 100mL/500mL

Storage: Please keep it sealed after opening and use, valid for 12 months.

Product Introduction:

Coomasylan G-250 dye, combined with protein in acidic solution, so that the dye's maximum absorption peak position (Imax), from 465nm to 595nm, in a certain concentration range, the determined absorbance value A595 is proportional to the protein concentration. Bradford method determination of protein concentration is not affected by most of the chemical substances in the sample, the concentration of merhydryl ethanol in the sample can be as high as 1M, the concentration of dithiothreitol can be as high as 5mM, but by a slightly high concentration of detergent, it is necessary to ensure that the concentration of SDS is less than 0.1%, Triton X-100 is less than 0.1%, And the Tween 20, 60 and 80 are below 0.06%. The BCA protein concentration assay kit produced by Solebao is recommended for samples containing detergents.

Self-supplied reagent:

PBS, BSA standard (5mg/mL)

Operating instructions (for informational purposes only) :

I. Microporous enzyme labeling method

1. Completely dissolved protein standard, take 10μL, dilute to 250μL, so that the final concentration is 0.2mg/mL. The standard product should also be diluted with the solution in which the protein sample to be measured is in. However, for the sake of simplicity, the standard can also be diluted with 0.9%NaCl or PBS.

2. Before use, please reverse and mix the 5×G250 dyeing solution 3-5 times, take 1mL of 5×G250 dyeing solution, add 4mL of double steaming water, and mix well to form 1×G250 dyeing solution. The 1×G250 dyeing solution can be stored at 4℃ for one week.

3. Add the standard product to the 96-well plate at the rate of 0,2,4,6,8,12,16,20 μl respectively, and add PBS diluent to make up to 20 μL.

4. Dilute the sample appropriately (preferably do several gradients, such as 2x, 4x, 8x dilution) and add 20 μL to the sample hole of the 96-well plate. Due to the error of the pipette when taking small amounts, the point in front of the standard line may not be very accurate, so as far as possible, let the sample point fall 1/2 behind the standard line.

5. Add 200 microliters of diluted 1 x G250 dye solution to each hole and let stand at room temperature for 3-5 minutes.

6. The absorbance of A595, or other wavelengths between 560-610nm, was determined with an enzyme-labeler.

7. The protein concentration in the sample was calculated according to the standard curve.

II. Spectrophotometer

if there is no enzyme label instrument, the dyeing reaction can be carried out in the centrifuge tube,

the reaction liquid is mixed and added to the colorimetric dish, and the light absorption value is determined by spectrophotometer.

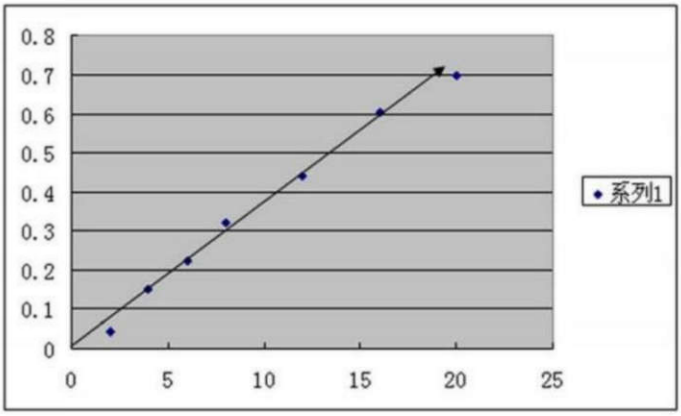
The steps are as follows:

1. Take eight (or more) clean 10mL centrifuge tubes and mark them with a number.
2. Take 100μLBSA and dilute it with PBS 2.4mL until the final concentration is 0.2mg/mL.
3. Before use, please reverse and mix the 5×G250 dyeing solution 3-5 times, take 10mL 5×G250 dyeing solution, add 40mL double steaming water, and mix well to form 1×G250 dyeing solution. The 1×G250 dyeing solution can be stored at 4℃ for one week.
4. Add the reagent according to the following table (5mL per well, the excess is used to clean the colorimetric dish)

Centrifuge tube number	1	2	3	4	5	6	7 (Sample tube 1)	8 (sample tube 2)	9 (sample tube 3)
Standard protein BSA	0 μL	100μL	200μL	300μL	400μL	500μL	500μL appropriately diluted sample 1	500μL appropriately diluted sample 2	……
PBS	500μL	400μL	300μL	200μL	100μL	0μL	0μL	0μL	0μL
1 x G250 stain solution	5mL	5mL	5mL	5mL	5mL	5mL	5mL	5mL	5mL

5. Measure the OD value after 3 minutes of reaction. For the accuracy of the experiment, a tube of dyeing solution can be added every 2 minutes, and a tube of OD value can be measured every 2 minutes. The following table:

Centrifugal tube number	1	2	3	4	5	6	7	8	……
Add dye solution (minutes)	0	2	4	6	8	10	12	14	……
Measure OD value	3	5	7	9	11	13	15	17	……



This picture is the measurement of Bole enzyme spectrometer 680, single wavelength, 570nm.

Reaction at room temperature for 3 minutes

Related products:

PC0001	*BSA standard (5mg/mL)*
PC0010	*Bradford protein concentration assay kit*
PC0021	*BCA reagent*
PC0030	*Lowry method protein concentration determination kit*
PC0020	*BCA method protein concentration determination kit*
R0010	*Highly efficient RIPA tissue/cell lysate*
PR1600	*prestain with low molecular weight protein MARKER*
R0050	*nuclear protein extraction kit*