Ready-to-use Bradford Protein Assay Kit

B1507335

Storage 2-8℃, Store in the dark.The BSA Standard is opened, it is recommended to aliquot and stored at -20℃ for long-term.

Shipping Low temperature transportation.

Introduction

Bradford Protein Assay Kit is a ready-to-use, stable traditional Bradford assay formulation for determining total protein concentration relative to a protein standard. The kit includes Bradford assay solution and ready-to-use diluted BSA standard solution, which was originally described by Bradford in 1976. When mixed with protein solutions, the color of the acidic Coomassie dye reagent changes from brown to blue in a manner proportional to the amount of protein present in the sample. Protein determination is performed by comparing the color response with that of a protein detection standard prepared as a known dilution series, typically as bovine serum albumin (BSA) or bovine gamma globulin (BGG). The simple procedure is suitable for almost any volume scale, including test tubes, cuvettes, and microplates. The protein detection is compatible with most salts, solvents, buffers, thiols, reducing substances, and metal chelators encountered in protein samples.

Product Features

1. The detection speed is extremely fast, with 10 to 80 samples completed in less than 10 minutes.
2. Convenient and efficient, it provides ready-to-use standards, eliminating the need for cumbersome dilution procedures.
3. It has high sensitivity, with a detection concentration lower limit of 25 μg/mL.
4. It has a good linear relationship within the concentration range of 50-2000 μg/mL.
5. The Bradford method for protein concentration determination is not affected by most chemical substances in the samples. However, it is affected by slightly higher concentrations of detergents. It is necessary to ensure Triton X-100 is less than 0.1%, and Tween 20 less than 0.008%. For samples containing detergents, it is recommended to use our BCA Protein Assay Kit (catalog number B1507333) or Ready-to-use Bradford Protein Assay Kit (Detergent Compatible) (catalog number B1509656). Please refer to the attached table of interferon substances for details.
6. Applicable to spectrophotometers or microplate readers.

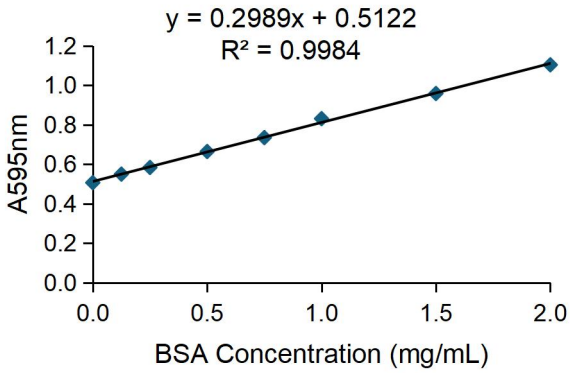

Figure 1. The actual measurement result of the protein standard curve of this kit. The data shown in the figure are for reference only; the actual detection results may vary slightly.

Kit Contents

B1507335	Components	1000T	storage temperature	Quantity Per Test
B1507335A	Bradford Assay Solution	250 mL	2-8℃, Store in the dark.	250 μL
B1507335B	BSA Standard (2.0 mg/mL)	1 mL	2-8℃	5 μL
B1507335C	BSA Standard (1.5 mg/mL)	1 mL	2-8℃	5 μL
B1507335D	BSA Standard (1.0 mg/mL)	1 mL	2-8℃	5 μL
B1507335E	BSA Standard (0.75 mg/mL)	1 mL	2-8℃	5 μL
B1507335F	BSA Standard (0.5 mg/mL)	1 mL	2-8℃	5 μL
B1507335G	BSA Standard (0.25 mg/mL)	1 mL	2-8℃	5 μL
B1507335H	BSA Standard (0.125 mg/mL)	1 mL	2-8℃	5 μL
B1507335I	BSA Standard (0 mg/mL)	1 mL	2-8℃	5 μL

Instruction for use

A. Microplate quantification assay (detection range = 50-2000μg/mL)

1. Preparation:

(1) Prepare the instruments: Turn on the microplate reader and set the reading wavelength (595nm).

(2) Prepare the Bradford Assay Solution: Take it out at 4℃ and let it warm up to room temperature. If it is left for 10 minutes.

2. Prepare the sample to be tested:

Dilute the sample protein to be tested with the same buffer/solution to make its estimated concentration fall within the linear range of the standard curve (usually 50-2000 μg/mL). Record the dilution factor, which will be used for subsequent calculation of the original

concentration. It is recommended to make 2-3 duplicate wells for each sample.

3. Add sample reaction:

(1) In the 96-well plate, add liquids according to the designed layout:

Standard curve wells: Add 5 μL of the corresponding concentration of standard protein solution (0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 mg/mL, etc.) to each well.

Blank control wells: Add 5 μL of buffer (0 mg/mL) to each well.

Sample wells: Add 5 μL of the diluted sample protein solution to each well.

(2) Add 250 μL of the Bradford Assay Solution to each well.

(3) Use a multi-channel pipette or a single-channel pipette to add the Bradford Assay Solution quickly and gently, avoiding the formation of bubbles as much as possible. After adding, seal the plate with a sealing film or gently tap it on the table several times, or use a plate shaker for a short period of time (low speed) to ensure that the liquid in the wells is fully mixed.

4. Incubation:

Place the sealed 96-well plate at room temperature and incubate for the specified time (e.g., 10 minutes).

5. Measure absorbance:

(1) After incubation, remove the plate from the water bath/oven and cool it to room temperature (about 10 minutes) at room temperature.

(2) Carefully dry the water vapor at the bottom of the plate. Place the plate in the microplate reader. Read the absorbance value of each well at 595 nm wavelength.

6. Data processing and drawing of standard curve:

(1) X-axis (horizontal axis): Standard protein concentration (μg/mL).

(2) Y-axis (vertical axis): Corrected absorbance value (Abs 595nm).

(3) Curve fitting:

The Bradford method (usually 50-2000 μg/mL) and the microplate microassay method (detection range = 1-25 μg/mL) usually show a good linear relationship within the appropriate detection range.

(4) Use software for linear regression to obtain the equation of the standard curve: Y = aX + b, where:

'Y' = corrected absorbance;

'X' = protein concentration (μg/mL);

'a' = slope;

'b' = Y-axis intercept.

(5) Calculate the correlation coefficient (R^2). An ideal standard curve should have an R^2 value very close to 1 (usually required > 0.99).

7. Calculation of sample concentration.

B. Micro-microplate quantification assay (detection range = 1-25μg/mL)

Take 150μL of the BSA standard and unknown samples to the corresponding labeled microplates, add 150μL of the Bradford Assay Solution to each well to mix and shake for 30

seconds, stop the shaking. For most cases, incubate the samples at room temperature for 10 minutes. Measure the absorbance value at 595 nm in the reading instrument, which can immediately determine the absorbance or measure within 2 hours. The data detected within 2 hours shows no significant change.

Matters needing attention

1. Reverting the G250 staining solution to room temperature before use is beneficial for enhancing the sensitivity of the detection. Before using the G250 staining solution, please invert it 3-5 times and mix well.
2. Some cations, such as K^+, Na^+, Mg^{2+}, $(NH_4)_2SO_4$, ethanol and other substances do not interfere with the measurement, but large amounts of detergents such as TritonX-100, SDS, etc. severely interfere with the measurement.
3. It is recommended that a standard curve be plotted each time the concentration of the protein sample is determined, in order to obtain accurate data. If the protein concentration is not within the range of the standard curve, please dilute the sample and measure it again.
4. For your safety and health, please wear laboratory coats and use disposable gloves during the operation.

Table 1. Interferon substance table

Substance	Compatible concentration	Substance	Compatible concentration
Buffers		Detergent	
Glycine	1M	Brij35	-
HEPES	0.2M	CHAPS	1%
MES	0.1M	NP-40	1%
MOPS	0.2M	SDS	-
Sodium citrate	0.2M	Triton X-100	-
Sodium phosphate	0.1M	Saccharides	
Sodium acetate	0.2M	Glucose	10mM
Tris	0.25M	Sucrose	1M
Salts		Chelating agents	
NaCl	1M	EDTA	10mM
Denaturing Agents		Reducing Agents	
GACl	4M	2-Mercaptoethanol	50μM
Urea	3M	DTT	1mM
Polar Compound		Other	
DMSO	10%	HCl	0.1M
Glycerol	10%	NaOH	0.1M