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Product Information

Bradford Protein Assay Kit

Catalog Number: P010

Unit Size: 2500 assays

Kit Contents

Component A: Bradford assay reagent, 500 mL;

Component B: BSA standard, 2 mg/mL, 10 mL.

Storage upon receipt:
- 2-8 °C
- Protect from light

Product Description

Bradford Protein Assay Kit is a quick and ready-to-use colorimetric assay for total protein quantitation. When coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm with a concomitant color change from brown to blue. Performing the assay is simple: combine a small amount of protein sample with the assay reagent, mix well, incubate briefly and measure the absorbance at 595 nm. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions, which are assayed alongside the unknown samples. Because the color response with coomassie in non-linear with increasing protein concentration, a standard curve must be completed with each assay.

Bradford assay is compatible with many of the salts, solvents, buffers, reducing chemicals and chelating agents often used in protein samples. Assays can be performed in tubes, or multi-well plate formats.

While the Bradford Reagent is compatible with reducing agents frequently used to stabilize proteins in solution, it is only compatible with low concentrations of detergent. If the protein samples to be assayed have detergent(s) in the buffer, use **BCA Protein Assay Kit** (Cat. No. P011).

Preparation of BSA Standard

Use Table 1 (see table in next page) as a guide to prepare a set of protein standards.

Test Tube Protocol

A. Standard Test Tube Protocol (Working range: 100-1500 µg/mL)

1. Pipette 30 µL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1.5 mL of Bradford reagent to each tube and mix well.
3. Incubate samples for 5 min at room temperature.
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Substrate the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595 nm measurements for each BSA standard vs, its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

B. Micro Test Tube Protocol (Working range: 1-25 µg/mL)

1. Pipette 1 mL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 1 mL of Bradford reagent to each tube and mix well.
3. Incubate samples for 5 min at room temperature.
4. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
5. Substrate the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595 nm measurements for each BSA standard vs, its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Protocol

A. Standard Microplate Protocol (Working range: 100-1500 µg/mL)

1. Pipette 5 µL of each standard or unknown sample into appropriate microplate wells.
2. Add 200 µL of Bradford reagent to each well and mix with plate shaker for 30 seconds.
3. Incubate plate for 5 min at room temperature.
4. Measure the absorbance at or near 595 nm with a plate reader.
5. Substrate the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595 nm measurements for each BSA standard vs, its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

B. Micro Microplate Protocol (Working range: 1-25 µg/mL)

1. Pipette 100 µL of each standard or unknown sample into appropriately labeled test tubes.
2. Add 100 µL of Bradford reagent to each well and mix with plate shaker for 30 seconds.
3. Incubate plate for 5 min at room temperature.
4. Measure the absorbance at or near 595 nm with a plate reader.
5. Substrate the average 595 nm measurement for the Blank replicates from the 595 nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average blank-corrected 595 nm measurements for each BSA standard vs, its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

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Table 1. Preparation of Diluted BSA Standards

Dilution Scheme for Standard Test Tube and Microplate Protocol (Working range: 100-1500 µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µL of Stock	2000 µg/mL
B	125 µL	375 µL of Stock	1500 µg/mL
C	325 µL	325 µL of Stock	1000 µg/mL
D	175 µL	175 µL of vial B dilution	750 µg/mL
E	325 µL	325 µL of vial C dilution	500 µg/mL
F	325 µL	325 µL of vial E dilution	250 µg/mL
G	325 µL	325 µL of vial F dilution	125 µg/mL
H	400 µL	100 µL of vial G dilution	25 µg/mL
I	400 µL	0	0 µg/mL (Blank)

Dilution Scheme for Micro Test Tube and Microplate Protocol (Working range: 1-25 µg/mL)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	2370 µL	30 µL of Stock	25 µg/mL
B	4950 µL	50 µL of Stock	20 µg/mL
C	3970 µL	30 µL of Stock	15 µg/mL
D	2500 µL	2500 µL of vial B dilution	10 µg/mL
E	2000 µL	2000 µL of vial D dilution	5 µg/mL
F	1500 µL	1500 µL of vial E dilution	2.5 µg/mL
G	5000 µL	0	0 µg/mL (Blank)

Related Products

Catalog No.	Product
P011	**BCA Protein Assay Kit**

References:

1. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254 (1976).

2. Stoscheck, CM. Quantitation of Protein. *Methods in Enzymology* 182: 50-69 (1990).