Bradford Reagent

Code	Description	Size
E530-1L	**Bradford Reagent**	1 L
M172-1L	**Bradford Reagent**	1 L
E535-KIT	**Protein Assay Bradford Method**	Kit
M173-KIT	**Bradford Method Protein Assay Kit**	Kit

General Information

VWR Life Science AMRESCO offers Biotechnology and Proteomics grade Bradford Reagents as solitary reagents or as part of kits with BSA standards. The Bradford Assay is utilized for rapid and colorimetric quantitation of proteins with microgram per milliliter sensitivity. The assay measures a shift in the absorption maximum that occurs upon complex formation between basic and aromatic amino acid residues with Coomassie® Brilliant Blue G-250 dye. Protein concentrations are determined in reference to the absorbances of protein standard dilutions, most commonly prepared using BSA. The assay is simple to perform and may be scaled from cuvette to microplate format.

Reference

Bradford, MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-54.

Storage/Stability

Store cold (4 - 8°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Supplied Materials

E530-1L	Bradford Reagent, 1 L
M172-1L	Bradford Reagent, 1 L
E535-KIT	Bradford Reagent, 1 L BSA Solution (0.5 mg/mL). 1.5 mL Sodium Chloride 0.15M, 200 mL
M173-KIT	Bradford Reagent, 1 L BSA Solution (0.5 mg/mL). 1.5 mL Sodium Chloride 0.15M, 200 mL

Protocol/Procedure

Protein Quantitation – 1 mL cuvette (Linear range: 0 µg/mL – 10 µg/mL)
Note: Gently mix Bradford Reagent, and then transfer the required volume to another container to equilibrate to room temperature before use.

1. Use the table below to prepare protein standards in triplicate using a 0.5 mg/mL BSA stock solution.

Standard Dilution	Volume 0.5 mg/mL BSA (µL)	Volume 0.15 M NaCl (µL)	Volume of Standard to Add Per Tube (µL)	BSA Per Tube (µg)
Blank	0	100	100	0
S1	5	95	100	2.5
S2	10	90	100	5
S3	15	85	100	7.5
S4	20	80	100	10

2. Add 1 mL Bradford Reagent to each standard dilution and mix. Allow to stand at room temperature for 2 minutes.
3. Measure absorbance at 595 nm using a 1 mL cuvette.
4. Generate a standard curve by plotting absorbance at 595 nm versus protein concentration.
5. For the unknown sample, repeat steps 1-4 using the unknown in place of the BSA. Use the standard curve as a reference to determine the concentration of the unknown.

Protein Quantitation – 96-Well Plate (Linear range: 0 µg/mL – 10 µg/mL)
Note: Gently mix Bradford Reagent, and then transfer the required volume to another container to equilibrate to room temperature before use.

1. Use the table below to prepare protein standards in triplicate using a 0.5 mg/mL BSA stock solution.

Standard Dilution	Volume 0.5 mg/mL BSA (µL)	Volume 0.15 M NaCl (µL)	Volume of Standard to Add Per Well (µL)	BSA Per Well (µg)
Blank	0	50	20	0
S1	2.5	47.5	20	0.5
S2	5	45	20	1
S3	7.5	42.5	20	1.5
S4	10	40	20	2

2. Pipette 20 µL of each standard into the wells of a 96-well plate.
3. Add 200 µL Bradford Reagent to each standard dilution and mix by pipetting. Allow to stand at room temperature for 2 minutes.
4. Measure absorbance at 595 nm using a plate reader.
5. Generate a standard curve by plotting absorbance at 595 nm versus protein concentration.
6. For the unknown sample, repeat steps 1-5 using the unknown in place of the BSA. Use the standard curve as a reference to determine the concentration of the unknown.

Frequently Asked Questions

Problem	Cause	Solution
Why are the standard dilution absorbances lower than expected?	Bradford Reagent not equilibrated to room temperature	Allow Bradford Reagent to warm to room temperature before use
	Bradford Reagent or standards stored improperly	Keep Bradford Reagent cold
	Standard dilutions not prepared properly	Follow the table in the protocol for standard preparation
	Measured absorbance at incorrect wavelength	Measure absorbance at 595 nm

Why are the sample absorbances lower than expected?	Protein purification is suboptimal	Optimize protein purification procedure
	Sample has molecular weight less than 3,000 Da	Try quantitation with a different method
Why are the samples dark blue?	Highly alkaline buffer raises the pH too high for the Bradford Reagent	Dialyze or dilute sample
Why is there a precipitate in the samples?	Samples contain detergent	Dialyze or dilute sample
	Dye aggregated because of insufficient mixing or because samples were standing too long	Mix samples with Bradford Reagent just prior to use

For Technical Support

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