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ab119216

Bradford Reagent

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes BFU05L.

View ab119216
Bradford Reagent datasheet:
www.abcam.com/ab119216
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For total protein quantitation

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Bradford Reagent (ab119216) is a quick and ready-to-use Coomassie-binding, colorimetric method for total protein quantitation in an environment containing up to 1% detergent (1% high protein range, 0.1% low protein range).

Bradford Reagent (ab119216) is an improvement over classical Bradford formulations which cannot tolerate detergent contamination of the protein samples. In addition, the Bradford Reagent shows excellent linearity for a defined range of protein concentrations and shows significantly less protein-to-protein variation than is observed with other Bradford-type Coomassie formulations.

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. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions, which are assayed alongside the samples with unknown protein concentrations.

2. Materials Supplied and Storage

Upon receipt store at +4°C. Discard any reagents that show discoloration or evidence of microbial contamination.

Item	Quantity	Storage temperature
Bradford Reagent	500 mL	+4°C

***Δ Note**: Reagent, containing Coomassie dyes, ethanol, phosphoric acid and solubilizing agents in water. (Caution: Phosphoric acid is a corrosive liquid.)*

3. Technical Considerations

3.1 Before use:

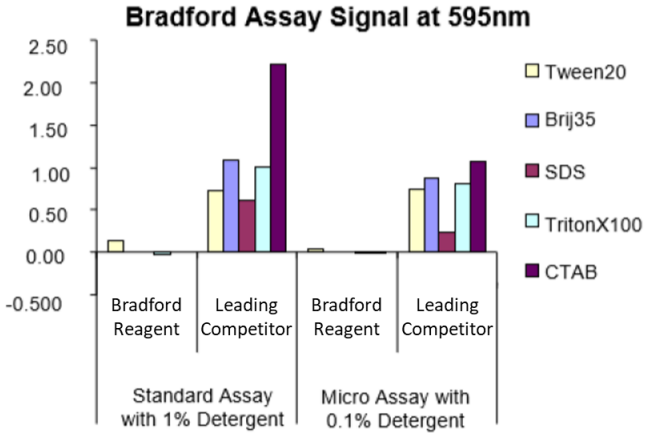
Mix the Bradford Reagent solution immediately before use by gently inverting the bottle several times (Do not shake the bottle to mix the solution). Remove the amount of reagent needed and equilibrate it to room temperature before use. It is good practice to mix the Bradford Reagent before dispensing and to mix each tube or plate immediately before measuring absorbances.

3.2 Protein Standards:

The intensity of the colour formation is protein dependent and is correlated to the basicity of the protein, in particular the number of positive charges on the protein (lysine, arginine and histidine), and hydrophobicity of the protein. A suitable standard will have a similar mol% positively charged residues as the target protein. For most proteins the content of basic amino acids ranges from 10 to 17 mol%1. BSA is the most commonly used reference standard in Bradford methods. Some useful standards in this range are given in the table below.

	Mol% positive residues
Hen egg white lysozyme	13.9
Bovine serum albumin (BSA)	16.5
Bovine Immunoglobin (IgG)	11.3
Bovine beta lactoglobulin	11.8

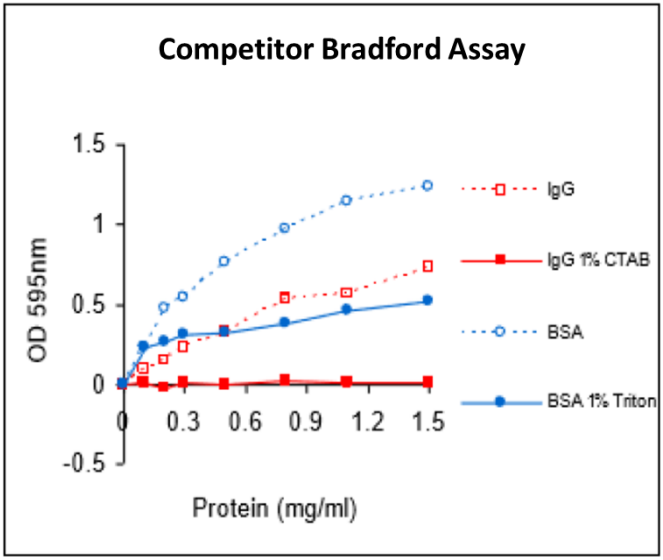
3.3 Detergent Compatibility:

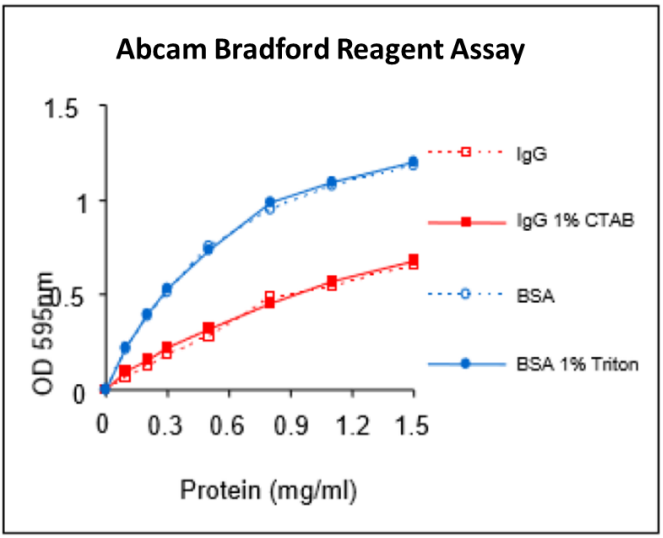


Comparison of Bradford Reagent Assay with leading competitor "Coomassie Protein Assay". The graph shows the average blank corrected A595 measurements for detergents.

Bradford Reagent is not affected by the detergents compared to Classic formulations which are subject to the high background noise at 595 nm in the presence of detergents.

Standard curves obtained with Bradford Reagent are unaffected by the presence of detergents. Standard curves obtained with classical Bradford formulation are significantly affected by the presence of detergents resulting in loss of sensitivity and inaccurate results.


Competitor Bradford Assay
1.5
1
0.5
0
-0.5
OD 595nm
0
0.3
0.6
0.9
1.2
1.5
Protein (mg/ml)
IgG
IgG 1% CTAB
BSA
BSA 1% Triton


Abcam Bradford Reagent Assay
1.5
1
0.5
0
OD 595nm
0
0.3
0.6
0.9
1.2
1.5
Protein (mg/ml)
IgG
IgG 1% CTAB
BSA
BSA 1% Triton

4. Assay Procedure

4.1 Make a dilution series of the chosen model protein in the range:
0.1 mg/mL – 1.5 mg/mL (high protein range)
or
1 µg/mL – 25 µg/ml (low protein range)

4.2 Mix the samples, standards and a blank (buffer, no protein) with Bradford Reagent.

<u>For 0.1 mg/mL – 1.5 mg/mL protein (high range):</u>
Sample / Reagent ratio: 1 / 15

For microtiter plate : 20 µL sample + 300 µL Bradford Reagent
For cuvette : 100 µL sample + 1.5 mL Bradford Reagent

<u>For 1 µg/mL – 25 µg/mL protein (low range):</u>
Sample / Reagent ratio: 1 / 1

For microtiter plate: 150 µL sample + 150 µL Bradford Reagent
For cuvette : 750 µL sample + 750 µL Bradford Reagent

*Δ **Note**: Preferably all samples, standards and blanks are prepared in triplicates.*

4.3 Read absorbance at 595 nm.

4.4 Subtract the average 595 nm measurement for the blank from the 595 nm measurements of all other individual standards and unknown samples. Plot the average blank-corrected 595 nm measurement for each standard vs. concentration. Use the slope of this standard curve to estimate the protein concentration of the unknown samples.

Technical Support

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