EZ-Bradford Assay Dye Reagent

Cat. No. DG-BRA500

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

The Bradford protein assay is a method for measuring protein concentration using Coomassie Brilliant Blue G-250(Coomassie). In an acidic environment, Coomassie is brown, but it turns blue when bound to proteins. This color change allows for the quantification of protein concentration.

The EZ-Bradford Assay Dye Reagent uses Coomassie to accurately measure protein concentration. It offers easy handling and a short reaction time, ensuring high efficiency in experiments.

▪ Contents

Component	Volume	Storage
Bradford Assay Dye Reagent	500 ㎖	4 ℃
Bradford Standard Solution (1.5 ㎎/㎖)	1 ㎖ X 10 ea	

* This product is intended for research use only and should not be used for human applications or diagnostic purposes.

▪ Storage and Stability

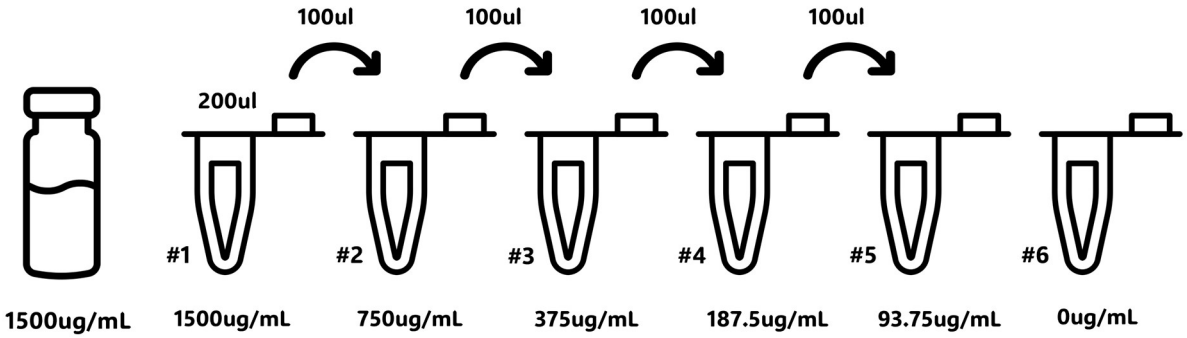
Store at 2 ~ 8 ℃ in a refrigerator. Stable for one year from the date of manufacture.

▪ General Protocol

- Allow all reagents to warm up to room temperature before starting the experiment.
- Dilute the Bradford Reagent 1 : 4 with distilled water (D.W.) before use.
- **Select the appropriate working range for your sample and proceed with the experiment.**

1-1. Spectrophotometer Measurement (Working range = 100 ~ 1,500 ㎍/㎖)

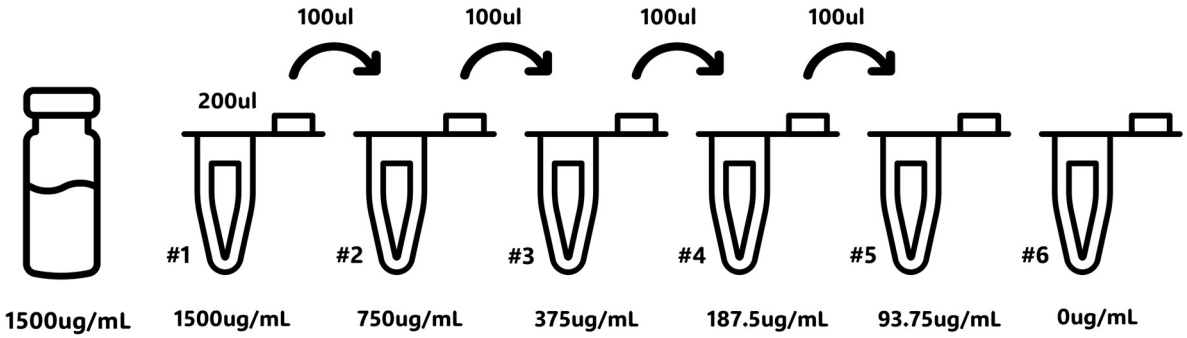
1) Prepare the standards as follows:
   (1) Prepare 6 vials (e-tubes) labeled #1 to #6.
   (2) Add 200 ㎕ of Bradford Standard Solution (1.5 ㎎/㎖) to vial #1. For vials #2 to #6, add 100 ㎕ of Distilled water (D.W.) to each.
   (3) Take 100 ㎕ from vial #1 (1.5 ㎎/㎖) and add it to vial #2. Mix thoroughly and repeat the serial dilution process up to vial #5. Vial 6 will serve as the blank.



2) Add 30 ㎕ of each prepared standard and sample into separate cuvettes.
3) Prepare the Diluted Bradford Assay Dye Reagent:
   Dilute the Bradford Assay Dye Reagent 1 : 4 with distilled water (D.W.). Prepare enough reagent for all samples, including standards, with some extra for convenience.
   Example) If measuring 10 samples:
   - Required volume calculation:
     {1.5 ㎖ x 6 ea(standard)} + {1.5 ㎖ x 10 ea(sample)} = 24 ㎖
   - Including extra volume:
     24 ㎖ + 1 ㎖(extra) = total 25 ㎖
   - Dilution method:
     Bradford Assay Dye Reagent 5 ㎖ + D.W 20 ㎖ (total 25 ㎖)
4) Add 1.5 ㎖ of the diluted Bradford Assay Dye Reagent to each cuvette containing standards or samples. Mix well.
5) Let the reaction proceed at room temperature for 10 minutes.
6) Use a spectrophotometer to measure the absorbance at 595 nm and 450 nm.

1-2. Microplate Reader Measurement (Working range = 100 ~ 1,500 ㎍/㎖)

1) Prepare Standards as follows.
   (1) Prepare 6 vials (e-tubes) labeled #1 to #6.
   (2) Add 200 ㎕ of Bradford Standard Solution (1.5 ㎎/㎖) to vial #1. For vials #2 to #6, add 100 ㎕ of Distilled water (D.W.) to each.
   (3) Take 100 ㎕ from vial #1 (1.5 ㎎/㎖) and add it to vial #2. Mix thoroughly and repeat the serial dilution process up to vial #5. Vial 6 will serve as the blank.



2) Add 5 ㎕ of each prepared standard and sample to the wells of a microplate.

- For accurate results, it is recommended to prepare at least two replicates for each standard and sample.

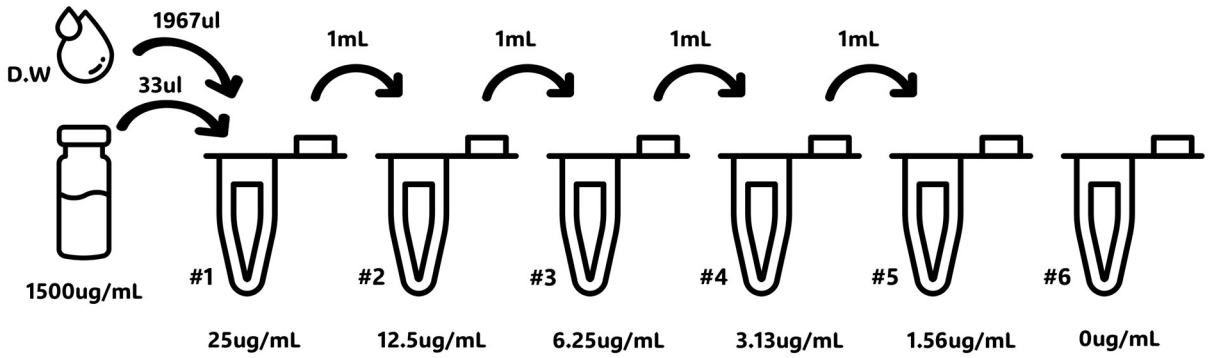
3) Prepare the Diluted Bradford Assay Dye Reagent:
   Dilute the Bradford Assay Dye Reagent 1 : 4 with distilled water (D.W.). Prepare enough reagent for all samples, including standards, with some extra for convenience.

   Example) If measuring 10 samples:
   - Required volume calculation:
     {250 ㎕ x 6 ea(standard)} + {250 ㎕ x 10 ea(sample)} = 8 ㎖
   - Including extra volume:
     8 ㎖ + 2 ㎖(extra) = total 10 ㎖
   - Dilution method:
     Bradford Assay Dye Reagent 2 ㎖ + D.W 8 ㎖ (total 10 ㎖)

4) Add 250 ㎕ of the diluted Bradford Assay Dye Reagent to each well and mix thoroughly.
5) Let the reaction proceed at room temperature for 10 minutes.
6) Use a Microplate Reader to measure the absorbance at 595 nm and 450 nm.
   ** If the measured O.D values are low, increase the volume of the standards and samples to 7 ~ 10 ㎕ and repeat the experiment.

1-3. Microplate Reader Measurement for Low-protein Concentration Samples (Working range = 1 ~ 25 ㎍/㎖)

1) Prepare the standards as follows:
   (1) Prepare 6 vials (e-tubes) labeled #1 to #6.
   (2) For vial #1, add 1967 ㎕ of D.W. and 33 ㎕ of Bradford Standard Solution (1.5 ㎎/㎖). For vials #2 to #6, add 1 ㎖ of D.W. to each.
   (3) Take 1 ㎖ from vial #1 (25 ㎍/㎖) and add it to vial #2. Mix thoroughly, then repeat the serial dilution process up to vial #5. Vial #6 serves as the blank.
   - After serial dilution, each vial (e-tube) will have a final volume of 1 ㎖.



2) Add 1 ㎖ of each sample to separate vials (e-tubes).
3) Prepare the Diluted Bradford Assay Dye Reagent:
   Dilute the Bradford Assay Dye Reagent 1 : 4 with distilled water (D.W.). Prepare enough reagent for all samples, including standards, with some extra for convenience.

   Example) If measuring 10 samples:
   - Required volume calculation:
     {1 ㎖ x 6 ea(standard)} + {1 ㎖ x 10 ea(sample)} = 16 ㎖
   - Including extra volume:
     16 ㎖ + 4 ㎖(extra) = total 20 ㎖
   - Dilution method:
     Bradford Assay Dye Reagent 4 ㎖ + D.W 16 ㎖ (total 20 ㎖)

4) Add 1 ㎖ of the diluted Bradford Assay Dye Reagent to each tube and mix thoroughtly.
5) Incubate the tubes at room temperature for 10 minutes.
6) Take 200 ㎕ from each tube and add it to a microplate, ensuring at least two replicates for each sample.
7) Use a Microplate Reader to measure the absorbance at 595 nm and 450 nm.

2. Calculation

1) Correct the values by calculating the ratio of O.D. at 595 nm to O.D. at 450 nm.

$$\Delta O.D_{595nm} \div \Delta O.D_{450nm}$$

2) Substract the blank value from all measurements for both standards and samples.
3) Use the Standard curve to determine the protein concentration in the samples.

▪ Notice

1. In the Bradford protein assay, various substances can interfere with the reaction, particularly by affecting the Coomassie Brilliant Blue G-250 dye, which can hinder accurate protein quantification. Below are the main interfering substances and suggested solutions.

Interfering Substance	Effect	Solution
Detergents	Ionic Detergents: Detergents containing SDS can cause errors in absorbance reading during Bradford measurements.	Use a lower amount or remove it completely before starting the experiment.
	Non-Ionic Detergents: Detergents such as Triton X-100 and Tween-20 interfere with dye binding, reducing color intensity.	
Salts	High concentrations of salts like ammonium sulfate or sodium chloride can affect protein structure or dye binding, making accurate measurement difficult.	Remove as much as possible using dialysis or desalting methods, or dilute the sample sufficiently before proceeding.
Buffers	Buffer solutions such as Tris, HEPES, and PBS can interfere with Coomassie Brilliant Blue G-250 dye at high concentrations, reducing accuracy. In particular, Tris Buffer absorbance measurements at 595 nm.	Use buffers at the lowest concentration and volume, or replace with bicarbonate buffer.
Reducing Agents	Reducing agents such as DTT(Dithiothreitol) and β-Mercaptoethanol interfere with protein quantification by reacting with Bradford reagent.	Use a lower concentration of reducing agents or remove them before proceeding.
Chelating Agents	Chelating Agents like EDTA can bind to metal ions and affect the interaction between proteins and the dye.	Use a buffer without EDTA or remove EDTA before proceeding.
Preservatives	Preservatives like NaN_3 can interfere with the Bradford dye reaction.	Use a solution without preservatives.
Glycerol	High concentrations of glycerol can after the absorbance of the Bradford reagent, causing measurements errors.	Remove glycerol or use sufficiently diluted samples.

2. When Coomassie Brilliant Blue G-250 binds to proteins, its absorbance is affected not only by the binding but also by the dye itself. As a result, using only the 595 nm measurement to plot a standard curve can lead to a low R^2 value. To address this, create the standard curve using a corrected value obtained by dividing the 595 nm O.D. by the 495 nm O.D.

3. The temperature of the reagent can affect the measurement. Ensure the product is fully warmed to room temperature before use.

4. If the measuring equipment does not have a 595 nm filter, absorbance can be measured at any available wavelength between 570 and 610 nm. However, note that using wavelengths other than 595 nm may result in a lower slope for the standard curve.

▪ Related Product

Product	Catalog No.	size
EZ-Western (Nano~mid picogram)	DG-W100	100 mL (A: 50 mL + B: 50 mL)
	DG-W250	250 mL (A: 125 mL + B: 125 mL)
	DG-W500	500 mL (A: 125 mL X 2 + B: 125 mL X 2)
EZ-Western Lumi Pico (Low picogram)	DG-WP100	100 mL (A: 50 mL + B: 50 mL)
	DG-WP250	250 mL (A: 125 mL + B: 125 mL)
	DG-WP500	500 mL (A: 125 mL X 2 + B: 125 mL X 2)
EZ-Western Lumi Pico Alpha (Low picogram)	DG-WPAL120	120 mL (A: 60 mL + B: 60 mL)
	DG-WPAL250	200 mL (A: 125 mL + B: 125 mL)
EZ-Western Lumi La (Mid femtogram, Long duration)	DG-WD100	100 mL (A: 50 mL + B: 50 mL)
	DG-WD200	200 mL (A: 100 mL + B: 100 mL)
EZ-Western Lumi Femto (Low femtogram)	DG-WF100	100 mL (A: 50 mL + B: 50 mL)
	DG-WF200	200 mL (A: 100 mL + B: 100 mL)
EZ-Western Membrane Tray	DG-WMT8	1 set (8 EA, 10 X 6 X 2 cm)
EZ-Western Stripping Buffer	DG-WSB500	500 mL
3-Color Regular Range Protein Marker, 10-245kDa	DG-PMC245	250 ㎕ x 2
3-Color Broad Range Protein Marker PLUS, 5-245kDa	DG-PMP245	250 ㎕ x 2
EZ-BCA Protein Quantification Kit	DG-BCA500	Reagent A : 500 mL Reagent B : 25 mL Standard Sol. : 1 mL X 10
EZ-Bradford Assay Dye Reagent	DG-BRA500	Reagent : 500 mL Standard Sol. : 1 mL X 10
EZ-Gel staining solution (without de-staining)	DG-GS1000	1000 mL