WSE-7510

EzBradford Protein Assay Kit

Instruction Manual

2025. Aug. 25 2nd ed.

1. Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time.

2. Purpose of use

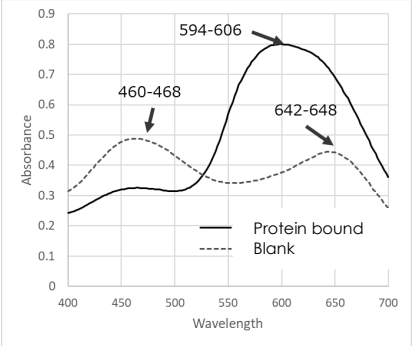
This product is a kit for quantifying total protein using the Bradford method*. It contains Bradford solution, standard proteins (BSA, BGG), and a protein dilution solution to reduce detergent interference. The approximate quantification range is 10–2000 µg/mL for the standard method and 1–50 µg/mL for the low-concentration method.
The Bradford method is a protein quantification method using the triphenylmethane dye Coomassie Brilliant Blue (CBB-G250). When CBB-G250 binds to proteins through non-covalent bonds, such as electrostatic interactions with basic amino acids (Arg, Lys, His) and N-terminal amino acids, and hydrophobic interactions with aromatic amino acids, the color (maximum absorption wavelength) changes from 465 nm (brown) to 595 nm (blue). Protein is quantified based on the absorbance at 595 nm.
※Bradford M. M., Analytical Biochemistry 72, 248-254 (1976)

3. Product Composition

Name	Volume	Quantity	Temperature
Bradford Reagent	500 mL	1	Refrigerated (2-10℃)
Protein Diluent	50 mL	1	Refrigerated /Room temp. (2-30℃)
BSA Standard	10 mL (2 mg/mL)	1	Refrigerated (2-10℃)
BGG Standard	10 mL (2 mg/mL)	1	Refrigerated (2-10℃)

Bradford Reagent is equivalent to 2500 samples when used at 200 µL/sample, and 500 samples when used at 1 mL/sample.
Protein Diluent is equivalent to 2500 samples when used at 20 µL/sample.

When CBB-G250 binds to a protein, the color (maximum absorption wavelength) changes from 465 nm to 595 nm.



4. Product configuration

Name	Main component
Bradford Reagent	CBB-G250, phosphoric acid, methanol, 1-hydroxyethane-1,1-bis(phosphonic acid) solution
Protein Diluent	Not provided
BSA Standard	Bovine serum albumin
BGG Standard	Bovine gamma globulin

This product contains substances subject to notification that exceed the exemption quantities specified under the PRTR Act, the Poisonous and Deleterious Substances Control Act, and the Industrial Safety and Health Act. For details, please download and refer to the SDS for this product from the ATTO website (https://www.atto.co.jp).

5. Storage

- This product is shipped refrigerated or at room temperature. After delivery, avoid direct sunlight and store at the appropriate temperature for each reagent. Unopened, it is stable within the expiration date (approximately one year from the date of manufacture).
- **Protein Diluent** may precipitate at low temperatures, but this does not affect its performance. If precipitation occurs, heat to 40-60°C and dissolve until all crystals have disappeared. If heating in a microwave, be careful not to heat the product too high.
- **Protein Diluent** can also be stored at room temperature (10-30°C).

6. Disposal

- Dispose of each reagent in accordance with your institution's disposal procedures. **Bradford Reagent**, in particular, contains phosphate, so please be careful when disposing of the liquid.
- Bottle Material
  Black Bottle Body: Polyethylene
  Lid: Polypropylene
  Gasket: Thermoplastic Elastomer
  White Bottle Body: Polypropylene
  Lid: Polypropylene

7. Items Required Other than This Product

- Measurement Container: Measurement cuvette, 96-well plate, etc.
- Measurement Equipment: Spectrophotometer, plate reader, etc.
- Pipette, tube, tips, etc.

8. Precautions for Use

- This product is pre-mixed. No additional reagent addition, mixing, or dilution is required.
- **Bradford Reagent** should be brought to room temperature before use and mixed by inversion until the solution is homogeneous. Avoid vigorous mixing and avoid foaming.
- **Bradford Reagent** is an acidic solution containing phosphoric acid, so handle with care.
- **Protein Diluent** may precipitate at low temperatures, but this does not affect its performance. If precipitation occurs, heat to 40-60°C to dissolve completely. If

heating in a microwave, be careful not to heat too high.

- When diluting proteins, use **Protein Diluent**, distilled water, PBS, or 0.9% NaCl. We recommend using **Protein Diluent** if the sample contains inhibitors such as detergents.
- Please note that the Bradford assay cannot quantify peptides under 3,000 Da. Also, please note that color development varies depending on the type of protein, which may affect the measurement.
- Pigment may deposit on quartz or glass cuvettes. After use, immerse in methanol or diluted chlorine bleach, then wash with glass detergent.
- BSA exhibits a stronger color development and a larger measured value than BGG or other proteins. We recommend using **BSA Standard** for samples containing albumin, such as plasma, and **BGG Standard** for other samples.
- Be sure to prepare a blank sample. Also, match the volume of the standard protein used to create the calibration curve and the measurement sample, and measure them in the same reaction system.
- When creating a calibration curve, use the average value after subtracting the blank value. Similarly, convert the measurement sample to the average value after subtracting the blank value.

9. How to Use

9-1. Preparation of Standard Proteins

1. Using the table below, prepare a dilution series (standard) by mixing the dilution solution with the **BSA standard** or **BGG standard**. The dilution solution uses **Protein Diluent**, distilled water, PBS, and 0.9% NaCl. A similar calibration curve can be obtained with a 1/2 dilution series starting from 1 mg/mL. The volume of each standard protein is 50 µL (100 µL for C and G). Adjust the volume accordingly.

Tube	Conc. (µg/mL)	Diluent	BSA/BGG Standard
A	1,500	25 µL	75 µL (Undil.)
B	1,000	50 µL	50 µL (Undil.)
C	750	50 µL	50 µL (A)
D	500	50 µL	50 µL (B)
E	250	50 µL	50 µL (D)
F	125	50 µL	50 µL (E)
G	25	80 µL	20 µL (F)
H	0	50 µL	0

*BSA exhibits better color development and higher measured values than BGG and other proteins. We recommend using the **BSA standard** for samples containing albumin, such as plasma, and the **BGG standard** for other samples.

2. When measuring low concentrations, use the table below to prepare a dilution series (low concentrations) by mixing the dilution solution with the **BSA standard** or **BGG standard**. The dilution solution uses **Protein Diluent**, distilled water, PBS, and 0.9% NaCl. Similar results can be obtained with a 1/2 dilution series starting from 50 µg/mL. The volume of each standard protein is 200 µL (A and G are 300 µL or more). Adjust the volume as needed.

9-2. Detergent-Free Sample (Standard)

(10-2,000 µg/mL)

Microwell Plate

1. Dispense 10 µL of each diluted standard protein solution and the measurement sample solution into each well of a 96-well plate. It is recommended to measure three wells in parallel (n=3).

Tube	Conc. (µg/mL)	Diluent	BSA/BGG Standard
A	50	585 µL	15 µL (Undil.)
B	25	100 µL	100 µL (A)
C	20	240 µL	160 µL (A)
D	15	140 µL	60 µL (A)
E	10	200 µL	200 µL (C)
F	5	200 µL	200 µL (E)
G	2.5	200 µL	200 µL (F)
H	0	200 µL	0

*The standard protein and measurement sample volumes can be reduced to 5 µL. In either case, ensure that the volumes of the standard protein and measurement sample are the same.

2. Add 200 µL of **Bradford Reagent** to each well and mix. Mix using a plate mixer or by gently tapping the edge of the plate.

*Before use, allow the **Bradford Reagent** to return to room temperature and mix by inversion to ensure a uniform solution. Be careful not to mix too vigorously or create bubbles.

*Be careful not to let the tip of the pipette come into contact with the plate or well. Also, be careful not to create bubbles.

3. The color reaction will begin immediately after adding **Bradford Reagent** and stabilize after a while (a few seconds to a few minutes). Measure the absorbance at 595 nm using a plate reader.

*The measured value will be stable for approximately one hour, depending on the sample solvent and protein concentration.

*Similar detection sensitivity and measured values will be obtained if the absorbance is in the 580-620 nm range.

Cuvette: 1 mL

1. Dispense 25 µL of each diluted standard protein solution and the measurement sample solution into 1.5 mL microtubes. We recommend measuring in duplicate (n=2).

*Adjust the volume appropriately depending on the size of the cuvette. Ensure that the volumes of the standard protein and measurement sample are the same.

*The reaction can also be performed directly in the cuvette.

2. Add 1 mL of **Bradford Reagent** to each of the above tubes and mix.

*Be careful not to let the tip of the pipette touch the tube wall or the liquid surface. Also, be careful not to create bubbles.

3. Measure the absorbance at a wavelength of 595 nm using a spectrophotometer.

*The measured value will be stable for approximately one hour, depending on the sample solvent and protein concentration.

*Similar detection sensitivity and measured values will be obtained if the absorbance is in the 580-620 nm range.

9-3. Detergent-Free Sample (Low Concentration)

(1-50 µg/mL)

Microwell Plate

1. Dispense 50 µL of each diluted standard protein solution and the measurement sample solution into each well of a 96-well plate. It is recommended to measure three wells in parallel (n=3).

2. Add 150 µL of **Bradford Reagent** to each well and mix. Mix using a plate mixer or by gently tapping the edge of the plate.

   *Before use, allow the **Bradford Reagent** to return to room temperature and mix by inversion to ensure a homogeneous solution. Be careful not to create bubbles by vigorously mixing.

   *Be careful not to let the tip of the pipette come into contact with the plate or well. Also, be careful not to create bubbles.

3. The color reaction will begin immediately after adding **Bradford Reagent** and will stabilize after a while (a few seconds to a few minutes). Measure the absorbance at 595 nm using a plate reader.

   *The measured value will be stable for approximately one hour, depending on the sample solvent and protein concentration.

   *Similar detection sensitivity and measured values will be obtained if the absorbance is in the 580-620 nm range.

Cuvette: 1 mL

1. Dispense 250 µL of each diluted solution of the standard protein and the measurement sample solution into 1.5 mL microtubes. We recommend measuring in duplicate (n=2).

   *Increase or decrease the volume as appropriate depending on the size of the cuvette. Make sure the volumes of the standard protein and measurement sample are the same.

   *The reaction can also be performed directly in the cuvette.

2. Add 750 µL of **Bradford Reagent** to each of the above tubes and mix.

   * Before use, allow the **Bradford Reagent** to return to room temperature and mix by inversion until the solution is homogenous. Avoid vigorous mixing to avoid creating bubbles.

   * Be careful not to let the tip of the pipette come into contact with the tube wall or the liquid surface. Also, be careful not to create bubbles.

3. Measure the absorbance at a wavelength of 595 nm using a spectrophotometer.

   * While the measured value depends on the sample solvent and protein concentration, it is stable for approximately one hour.

   * Similar detection sensitivity and measurement values can be obtained if the absorbance is in the 580-620 nm range.

9-4. Sample Containing Surfactant (Standard)
(10-2,000 µg/mL)

Microwell Plate

1. Dispense 10 µL of each diluted standard protein solution and the measurement sample solution into each well of a 96-well plate. It is recommended to measure three wells in parallel (n=3).

   *If the sample contains inhibitors such as high concentrations of surfactants, dilute appropriately with **Protein Diluent**. For information on the effects of coexisting substances in the sample, please refer to the table at the end of this manual.

2. Add 20 µL of **Protein Diluent** to each well and mix. Mix using a plate mixer or by gently tapping the edge of the plate. Add this to the standard protein as well.

   *Be careful not to let the tip of the pipette come into contact with the plate or well. Also, be careful not to create bubbles.

   *Add **Protein Diluent** in a volume 2-3 times the volume of the measurement sample. Ensure that the volumes of the standard protein and measurement sample are the same.

3. Add 200 µL of **Bradford Reagent** to each well and mix. Mix using a plate mixer or by gently tapping the edge of the plate.

   *Before use, allow the **Bradford Reagent** to return to room temperature and mix by inversion or other means to ensure a uniform solution. Be careful not to create bubbles by mixing vigorously.

4. The color reaction begins immediately after adding **Bradford Reagent** and stabilizes after a while (a few seconds to a few minutes). Measure the absorbance at 595 nm using a plate reader.

   *The measured value will be stable for approximately one hour, depending on the sample solvent and protein concentration.

   *Similar detection sensitivity and measured values will be obtained if the absorbance is in the 580-620 nm range.

Cuvette: 1 mL

1. Dispense 25 µL of each diluted standard protein solution and the measurement sample solution into 1.5 mL microtubes. We recommend measuring in duplicate (n=2).

   *Adjust the volume appropriately depending on the size of the cuvette. Ensure that the volumes of the standard protein and measurement sample are the same.

   *Direct reaction in the cuvette is also possible.

2. Add 50 µL of **Protein Diluent** to each of the above tubes and mix.

   *Be careful not to let the tip of the pipette come into contact with the tube wall or the liquid surface. Also, be careful not to create bubbles.

   *Add 2-3 times the volume of **Protein Diluent** as the measurement sample. Ensure the volumes of the standard protein and the measurement sample are the same.

3. Add 1 mL of **Bradford Reagent** to each of the above tubes and mix.

   *Equilibrate the **Bradford Reagent** to room temperature before use and mix by inversion or other means until the solution is homogenous. Be careful not to create bubbles by vigorously mixing.

   *Be careful not to let the tip of the pipette come into contact with the tube wall or the liquid surface. Also, be careful not to create bubbles.

4. Measure the absorbance at a wavelength of 595 nm using a spectrophotometer.

   *Measurements are stable for approximately 1 hour, depending on the sample solvent and protein concentration.

   *Similar detection sensitivity and measurement values can be obtained if the absorbance is in the 580-620 nm range.

9-5. Samples Containing Surfactants (Low Concentration)
(5-50 µg/mL)

Microwell Plate

1. Dispense 50 µL of each diluted standard protein solution and the measurement sample solution into each well of a 96-well plate. We recommend measuring three wells in parallel (n=3).

   *If the sample contains inhibitors such as high concentrations

of surfactants, dilute them appropriately with **Protein Diluent**. For information on the effects of coexisting substances in the sample, refer to the table at the end of this manual.

2. Add 100 µL of **Protein Diluent** to each well and mix. Mix using a plate mixer or by gently tapping the edge of the plate. Add this to the standard protein as well.

   *Be careful not to touch the tip of the tip to the plate or well. Also, be careful not to create bubbles.

   *Add 2-3 times the volume of **Protein Diluent** as the measurement sample. Make sure the volumes of the standard protein and measurement sample are the same.

3. Add 150 µL of **Bradford Reagent** to each well and mix. Mix using a plate mixer or by gently tapping the edge of the plate.

   * Before use, bring the **Bradford Reagent** to room temperature and mix by inversion to ensure a homogeneous solution. Be careful not to mix vigorously or create bubbles.

4. The color reaction begins immediately after adding the **Bradford Reagent** and stabilizes after a while (a few seconds to a few minutes). Measure the absorbance at 595 nm using a plate reader.

   * The measured value will be stable for approximately one hour, depending on the sample solvent and protein concentration.

   * Similar detection sensitivity and measured values will be obtained if the absorbance is in the 580-620 nm range.

Cuvette: 1 mL

1. Dispense 250 µL of each diluted standard protein solution and the measurement sample solution into 1.5 mL microtubes. It is recommended to measure in duplicate (n=2).

   *Increase or decrease the volume appropriately depending on the size of the cuvette. Ensure that the volumes of the standard protein and measurement sample are the same.

   *Direct reaction in the cuvette is also possible.

2. Add 500 µL of **Protein Diluent** to each of the above tubes and mix.

   *Be careful not to let the tip of the pipette touch the tube wall or the liquid surface. Also, be careful not to create bubbles.

   *Add 2-3 times the volume of **Protein Diluent** as the measurement sample. Ensure that the volumes of the standard protein and measurement sample are the same.

3. Add 750 µL of **Bradford Reagent** to each of the above tubes and mix.

   *Be careful not to let the tip of the pipette touch the tube wall or the liquid surface. Also, be careful not to create bubbles.

4. Measure absorbance at a wavelength of 595 nm using a spectrophotometer.

   *Measured values are stable for approximately 1 hour, although this depends on the sample solvent and protein concentration.

   *Similar detection sensitivity and measured values will be obtained if the absorbance is in the 580-620 nm range.

10. Overview of the Bradford Reaction

The table below briefly summarizes the sample and reagent volumes required for the Bradford reaction, as well as the method.

	Standard		Low range		Detergent contained (Standard)		Detergent contained (Low range)	
Container	Plate	Cuvette	Plate	Cuvette	Plate	Cuvette	Plate	Cuvette
Sample volume (Standard protein)	10 µL	25 µL	50 µL	250 µL	10 µL	25 µL	50 µL	250 µL
Protein Diluent	-		-		20 µL	50 µL	100 µL	500 µL
Bradford Reagent	200 µL	1 mL	150 µL	750 µL	200 µL	1 mL	150 µL	750 µL
Reaction temperature	Room temperature		Room temperature		Room temperature		Room temperature	
Reaction time	5 sec～10 min (Stable for 1h)		5 sec～10 min (Stable for 1h)		5 sec～10 min (Stable for 1h)		5 sec～10 min (Stable for 1h)	
Wavelength	595 nm		595 nm		595 nm		595 nm	
Sensitivity	10-2,000 µg/mL		1-50 µg/mL		10-2,000 µg/mL		5-50 µg/mL	

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