WSE-7520

EzBCA Protein Assay Kit Instruction manual

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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 BCA method * . It contains BCA solutions (A and B), standard proteins (BSA, BBGG) and a **Pretreatment Agent** and **Pretreatment Solution** to reduce interference from reducing agents. The approximate quantitative range is 15 to 2000 µg/mL for the standard method and 3 to 100 µg /mL for the low-concentration method. µg/mL.

The BCA method is an improved version of the Lowry method. Peptides and proteins consisting of three or more amino acids form complexes with $Cu2^{+}$ under alkaline conditions. The $Cu2^{+}$ is reduced to Cu^{+} by the reducing amino acids (Cys, Tyr, Trp) contained in the protein, and the released Cu^{+} then coordinates with two BCA molecules to form a complex. The amount of protein is quantified from the absorbance at 562 nm, where this complex exhibits strong absorption.

3. Product Configuration

Name	Volume	Quantity	Storage temperature
BCA reagent A	500 mL	1 bottle	Room temperature (15-30 ℃) (or refrigerate)
BCA reagent B	12 mL	1 bottle	Room temperature (15-30 ℃) (or refrigerate)
Pretreatment Solution	50 mL	1 bottle	Refrigerate (2-10 ℃)
Pretreatment Agent	800 mg	1 bottle	Refrigerate (2-10 ℃)
BGG Standard	10 mL (2 mg/mL)	1 bottle	Refrigerate (2-10 ℃)
BGG Standard	10 mL (2 mg/mL)	1 bottle	Refrigerate (2-10 ℃)

BCA solution is BCA Reagent Mix **BCA reagent A** and **BCA reagent B** at a 50:1 ratio. This solution is equivalent to 2500 samples when used at 200 µL/sample, or 500 samples when used at 1 mL/sample. Protein **Pretreatment Solution** is prepared by adding 28 mg of **Pretreatment Agent** to **Pretreatment Solution**. Dissolve in 1 mL for use. When used at 12.5 µL per sample, this is equivalent to approximately 2200 samples.

4.Composition

Name	Main component
BCA reagent A	Bicinchoninic acid disodium salt
BCA reagent B	Copper (II) sulfate pentahydrate
Pretreatment Solution	Not disclosed
Pretreatment Agent	Iodoacetamide
BGG 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 products are stable within their expiration date (approximately one year from the date of manufacture).
- **Pretreatment Solution** may precipitate when stored 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 raise the temperature too much.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your institution. In particular, please be careful when disposing of **BCA reagent B**, as it is a heavy metal solution containing copper sulfate.
- Bottle Material

Black bottle	body: polyethylene Lid: Polypropylene Packing: 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.
- Pipetman, tubes, tips, etc.

8. Precautions for use

- **BCA reagent A** Before use, mix the solution by inverting it to make it uniform.
- **Pretreatment Solution** may precipitate at low temperatures, but it does not affect the performance. If it occurs, heat to 40-60 °C and dissolve until all crystals have disappeared. If heating in a microwave, be careful not to raise the temperature too much.
- **BCA reagent A** is an alkaline solution with a pH of 11 or higher, so please handle it with care, such as by avoiding the use of glass containers.
- **BCA reagent B** is a heavy metal solution containing sulfate copper, so handle it with care. Do not dispose of directly into the drain/sink.
- When diluting proteins, use distilled water, PBS, or 0.9% NaCl.
- Pigments may be deposited on quartz or glass cuvettes. After using it, soak them in methanol or diluted chlorine bleach, then clean with glass detergent.
- **Pretreatment Agent** is irritating to the skin and eyes, highly reactive, and decomposes when exposed to light or heat, so please be careful when storing and handling.
- The BCA method has little difference in measurement values depending on the type of protein, and either BSA or BGG can be used as the standard protein. We recommend using the BSA standard for samples containing albumin, such as plasma, and the BGG standard for samples such as antibodies.
- Be sure to prepare a blank sample. Also, the volumes of the standard protein used to create the calibration curve, and the measurement sample must be the same, and they must be measured in the same reaction condition.
- When creating a calibration curve, use the average value after subtracting the blank value. The measurement samples are converted based on the average value after subtracting the blank value.

9.How to use

9-1. Preparation of Standard Proteins

1. Refer to the table below and mix the diluted solution with **BSA Standard** or **BGG Standard** to make a dilution series (standard). The dilution solutions used are distilled water, PBS, or 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 100 µL (C is 200 µL) . Adjust the volume accordingly.

Tube	Concentration (µg /mL)	Dilute solution (distilled water, etc.)	BSA/BGG Standard
A	100	380 µL	20 µL
B	75	25 µL	75 µL (A)
C	50	100 µL	100 µL (A)
D	225	100 µL	100 µL (C)
E	25	160 µL	40 µL (A)
F	10	100 µL	100 µL (E)
G	5	100 µL	100 µL (F)
H	0	100 µL	0

2. When measuring low concentrations, refer to the table below and mix the diluted solution with **BSA Standard** or **BGG Standard** to make a dilution series (low concentrations). The dilution solutions used are distilled water, PBS, or 0.9% NaCl. Similar results were obtained with a 1/2 dilution series starting from 100 µg/mL. The volume of each standard protein is 100 µL (A, D, G: 150 µL or more). Adjust the volume accordingly.

Tube	Concentration (µg /mL)	Dilute solution (distilled water, etc.)	BSA/BGG Standard
A	1,500	50 µL	150 µL
B	1,000	100 µL	100 µL
C	750	100 µL	100 µL (A)
D	500	100 µL	100 µL (B)
E	250	100 µL	100 µL (D)
F	125	100 µL	100 µL (E)
G	25	80 µL	20 µL (F)
H	0	100 µL	0

9-2. Preparation of Working Solution

1. Prepare the **BCA Working Solution** by mixing **BCA reagent A** and **BCA reagent B** in a 50 : 1 ratio. For example, to prepare the amount required for one 96 -well plate, add 0.8 mL of **BCA reagent B** to 40 mL of **BCA reagent A** and mix.
   ∗ When **BCA reagent B** is added, it may appear to precipitate for a moment, but it will dissolve immediately upon mixing, forming a pale green solution.
   ∗ Prepare the **Working Solution** immediately before use and do not store it.

9-3. Sample without reducing agent (standard)

(15~2,000µg / mL)

Microwell plate

1. 25 µL of each diluted standard protein solution and the measurement sample solution into each well of a 96 -well plate. We recommend measuring triplicate wells (n = 3).
2. Add 200 µL of **Working Solution** to each well and mix by using a plate mixer or by gently tapping the edge of the plate.
   ∗ Be careful not to let the tip of the tip come into contact with the plate or well. Also, be careful not to create bubbles.
3. Cover with a lid or plastic wrap and incubate at 37 ℃ for 30 minutes. After the reaction, return to room temperature.
4. Measure the absorbance at 562 nm using a plate reader.
   ∗ Similar detection sensitivity and measurement values can be obtained if the absorbance is in the range of 540 to 570 nm.

Cuvette: 1 mL

1. Dispense 50 µL of each diluted standard protein solution and the measurement sample solution into 1.5 mL microtubes. We recommend measuring it in duplicate (n = 2).
   ∗ Please increase or decrease the volume as appropriate depending on the size of the cuvette. In this case, make sure that the volumes of the standard protein and the

measurement sample are the same.

2. Add 1 mL of **Working Solution** to each of the above tubes and mix.
   ∗ Be careful not to let the tip of the tip come into contact with the tube wall or the liquid surface. Also, be careful not to create bubbles.
3. Incubate at 37 ℃ for 30 minutes. After the reaction, return to room temperature.
4. Measure the absorbance at 562 nm using a spectrophotometer.
   ∗ Similar detection sensitivity and measurement values can be obtained if the absorbance is in the range of 540 to 570 nm.

9-4. Sample without reducing agent (low concentration)
(3~100μg / mL)

Microwell plate

1. 25 μL of each diluted standard protein solution and the measurement sample solution into each well of a 96 -well plate. We recommend measuring triplicate wells (n = 3).
2. Add 200 μL of **Working Solution** to each well and mix by using a plate mixer or by gently tapping the edge of the plate.
   ∗ Be careful not to let the tip of the tip come into contact with the plate or well. Also, be careful not to create bubbles.
3. Cover with a lid or plastic wrap and heat at 60 ℃ for 30 to 60 minutes. After the reaction, return to room temperature.
   ∗ Increasing the reaction time may increase the detection sensitivity.
4. Measure the absorbance at 562 nm using a plate reader.
   ∗ Similar detection sensitivity and measurement values can be obtained if the absorbance is in the range of 540 to 570 nm.

Cuvette: 1 mL

1. Dispense 50 μL of each diluted standard protein solution and the measurement sample solution into 1.5 mL microtubes. We recommend measuring it in duplicate (n = 2) .
   ∗ Please increase or decrease the volume as appropriate depending on the size of the cuvette. In this case, make sure that the volumes of the standard protein and the measurement sample are the same.
2. Add 1 mL of **Working Solution** to each of the above tubes and mix.
   ∗ Be careful not to let the tip of the tip come into contact with the tube wall or the liquid surface. Also, be careful not to create bubbles.
3. Heat at 60 ℃ for 30 to 60 minutes. After the reaction, return to room temperature.
   ∗ Increasing the reaction time may increase the detection sensitivity.
4. Measure the absorbance at 562 nm using a spectrophotometer.
   ∗ Similar detection sensitivity and measurement values can be obtained if the absorbance is in the range of 540 to 570 nm .

9-5. Samples containing reducing agents (pretreatment)

<Preparing the **Pretreatment Agent**>

1. Bring **the Pretreatment Solution** and **Pretreatment Agent to room temperature.**
2. Weigh out 28 mg of **Pretreatment Agent** and dissolve it in 1 mL of **Pretreatment Solution** to create the **Pretreatment Reagent .**
   ∗ The amount of Pretreatment Reagent required is equal to the amount of standard protein for the calibration curve and the amount of measurement sample.
   ∗ When using a 96-well plate and measuring n=3, 40 μL is required per sample (3 wells). When using cuvettes (1 mL) and measuring n=2, 55 μL is required per sample. 1 mL allows for pretreatment of 25 samples (75 wells), or 18 samples.
   ∗ **Pretreatment Reagent** can be stored in a refrigerator, protected from light, for one week.

Pretreatment reaction

1. Dispense 40 μL of each diluted standard protein solution and the measurement sample solution into 1.5 mL microtubes (60 μL if measuring in a cuvette).
   ∗ When using a 96-well plate and measuring n=3, 40 μL is required per sample (3 wells). When using cuvettes (1 mL) and measuring n=2, 55 μL is required per sample.
   ∗ Reactions within the wells of a 96 -well plate or cuvette used for measurement may cause uneven reactions.
2. Add an equal volume of **Pretreatment Reagent** (40 μL or 60 μL) to each tube and mix thoroughly.
   *Mix using a vortex mixer or by tapping. Spin down if necessary.
3. Collect the reaction mixture at the bottom of the tube by spinning down and incubate at 37 °C for 10 to 15 minutes in the dark.

9-6. Sample containing reducing agent (measurement)
(125~2,000μg / mL)

Microwell Plate

1. Dispense 25 μL of each diluted standard protein solution and the measurement sample solution (pretreated as described in 9-4) into each well of a 96 -well plate. We recommend measuring three wells in parallel (n = 3).
2. Add 250 μL of **Working Solution** to each well and mix by using a plate mixer or by gently tapping the edge of the plate.
   ∗ Be careful not to let the tip of the pipette tip come into contact with the plate or well. Also, be careful not to create bubbles.
3. Cover with a lid or plastic wrap and incubate at 37 ℃ for 30 minutes. After the reaction, return to room temperature.
4. Measure the absorbance at 562 nm using a plate reader.
   ∗ Similar detection sensitivity and measurement values can be obtained if the absorbance is in the range of 540 to 570 nm.

Cuvette: 1 mL

1. Dispense 50 μL of each diluted standard protein solution and the measurement sample solution (pretreated as described in 9-4) into 1.5 mL microtubes. It is

recommended to measure in duplicate (n = 2).

∗ Please increase or decrease the volume as appropriate depending on the size of the cuvette. In this case, make sure that the volumes of the standard protein and the measurement sample are the same.

2. Add 1 mL of **Working Solution** to each of the above tubes and mix.

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

3. Incubate at 37 ℃ for 30 minutes. After the reaction, return to room temperature.
4. Measure the absorbance at 562 nm using a spectrophotometer.

   ∗ Similar detection sensitivity and measurement values can be obtained if the absorbance is in the range of 540 to 570 nm.

10. Overview of the BCA reaction

The table below shows the amount of the reagent required for the measurement reaction. This is a method for preparing the **Working Solution** and the **Pretreatment Reagent** required for the pretreatment reaction.

Working Solution **Working Solution**	**BCA reagent A**	**BCA reagent B**
	40 mL	0.8 mL

Prepared **Pretreatment Solution** **Pretreatment Reagent**	**Pretreatment Agent**	**Pretreatment Solution**
	28 mg	1 mL

The table below briefly summarizes the measurement samples and reagent volumes required for the reaction with this reagent, as well as the method.

	Standard		Low concentration measurement		Contains reducing agents	
Measuring container	plate	cuvette	plate	cuvette	plate	cuvette
Sample amount (pretreatment)					40 µL (enough for 3 wells)	55 µL (n=2)
Pretreatment Reagent					40 µL (enough for 3 wells)	55 µL (n=2)
Pretreatment reaction temperature					37 ℃	
Pretreatment reaction time					10~15 minutes	
Sample amount (measurement)	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL
Working Reagent	200 µL	1 mL	200 µL	1 mL	250 µL	1 mL
Reaction temperature	37 ℃		60 ℃		37 ℃	
Reaction time	half an hour		30-60 minutes		half an hour	
Measurement wavelength	562 nm		562 nm		562 nm	
Detection sensitivity	15-2,000 µg /mL		3-100 µg /mL		125-2,000 µg /mL	

The volumes of sample and **Pretreatment Reagent** used for pretreatment listed in the table above are the required volumes when measuring n=3 in a 96-well plate and n=2 in a cuvette (1 mL). Please increase or decrease the volumes as appropriate.

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