

AE-1476 EzBlock BSA operating instructions

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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 used as a blocking reagent for Western blotting, ELISA, and immunostaining of cells and tissues, and as a diluent for antibodies.

3. Product configuration

Name	Volume	Quantity
EzBlock BSA	200 mL	1
Tween 20	10 mL	1

4. Composition

Name	Main component
EzBlock BSA	Tris (Hydroxymethyl)aminomethane Sodium chloride Albumin derived from bovine serum (Cohn Fraction V) Preservative (This is 5 times concentrated stock solution.)
Tween 20	Tween 20 (This is 100 times concentrated.)

This product does not fall under the GHS classification criteria. For details, please download the SDS for this product from the ATTO website (<https://www.atto.co.jp/>).

5. Storage

- **EzBlock BSA** should be stored refrigerated (2-10 °C). Unopened, it is stable until the expiration date
- Do not mix stock solution of **EzBlock BSA** with another blocking reagent.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your affiliated institution.
- Material
Bottle: High density polyethylene Lid: Polypropylene
Tube Main body : PET Lid : Polypropylene

7. Items required other than this product

- Distilled water

8. Precautions for use

- This product is 5 times concentrated stock solution of blocking reagent whose main component is albumin derived from bovine serum. When you use it, dilute it in accordance with usage.
- 1 bottle of **EzBlock BSA** is available for blocking about 20 membranes of mini slab gel size.
- It doesn't include sodium azide inhibiting activity of HRP. Also preservative included in this product doesn't inhibit activity of HRP and ALP.

9. How to use

A. When used as a blocking reagent for western blotting

1. **Dilute EzBlock BSA 5times with distilled water.** The minimum amount is 0.65 mL/cm² . For mini slab gel size , approximately 50 mL is required. To make 50 mL of working solution, use **EzBlockBSA** 10 mL of stock solution mix with 39.5 mL of distilled water .
2. Add 1% amount of **Tween 20** to working solution of **EzBlock BSA**. In the case of the above solution (49.5mL), add 0.5mL **Tween 20** to it.
※When making working solution of **EzBlock BSA**, another blocking reagent can be added.
※When working solution of **EzBlock BSA** may be diluted more, use **EzTBS(+Tween20)** or TBS-T.
3. Pour the above **EzBlock BSA** working solution into a container large enough to allow the blotting membrane to move freely in the shaking direction .
1. Immerse the blotting membrane in **EzBlock BSA** working solution and shake at room temperature for 30 to 60 minutes.
※Longer blocking times (over 1 hour) can cause overblocking.

B. When use as a blocking reagent for ELISA

1. **Dilute EzBlock BSA 5times with distilled water.** When you make 50mL working solution, mix 10mL stock solution of **EzBlock BSA** with 40mL distilled water.
2. Add 100-300 μ L of the working solution to each well of 96 well plate.
3. Incubate it at room temperature for 30-60min.

※If blocking reagent is removed from the plate, ELISA plate after blocking can be dried and then stored in refrigerating chamber. The preservation term is varied by the stability of protein or antibody coated on the ELISA plate.

※It is necessary to add **Tween 20** depending on the materials of ELISA plate. Please contact the manufacturer of your ELISA plate.

※If blocking reagent including **Tween 20** is always used, add all amount of **Tween 20** attached to this product to the bottle of **EzBlock BSA** after opening the package.

1. After you receive this product, add the whole amount of **Tween 20** attached to the product to the bottle of **EzBlock BSA** before using it for the first time. If **Tween 20** is added, check a box "**Tween 20** ☐
2. Dilute stock solution of **EzBlock BSA** including **Tween 20** 5times with distilled water for making working solution of **EzBlock BSA**. Dilute 10.5mL the stock solution including **Tween 20** in measuring cylinder to 50mL total with distilled water.
3. In accordance with Usage A-D, use it as blocking reagent or antibody diluent.

C. When use as a blocking reagent for immunostaining

1. **Dilute EzBlock BSA 5times with distilled water.** When you make 50mL working solution, mix 10mL stock solution of **EzBlock BSA** with 40mL distilled water.
2. Immerse slide of cell and tissue section in working solution of **EzBlock BSA**. Or, add enough **EzBlock BSA** working solution on top of the sample to cover it completely (approximately 100 μ L for a 1 cm x 1 cm area).
3. Incubate it at room temperature for 30-60min.

※It is necessary to add **Tween 20** depending on the state of cell and tissue, and materials of slide plate. Confirm blocking condition of each experiment.

10. Additional information

Even with the same blotting protocol, slight differences in technique can greatly affect the results. Tips and tricks are also important for obtaining optimal results. Please read the "Tips for Western Blotting" which can be downloaded from the Atto website.
[https:// www.atto.co.jp/](https://www.atto.co.jp/)

D. When use as an antibody diluent

1. **Dilute EzBlock BSA 5times with distilled water.** When you make 50mL working solution, mix 10mL stock solution of **EzBlock BSA** with 39.5mL distilled water.
2. Add 1% amount of **Tween 20** to working solution of **EzBlock BSA**. In the case of the above solution (49.5mL), add 0.5mL **Tween 20** to it.

※It is not necessary to add **Tween 20** depending on the antibody.

※When working solution of **EzBlock BSA** may be diluted more, use **EzTBS(+Tween20)** or TBS-T.

※In general, if diluent for western blotting doesn't include adequate amount of salt and surfactant, background tends to increase.



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