AE-1476 *EzBlock BSA* *Instruction Manual*

July 17th, 2012 Ver. 3

1. Safety precaution

Before using this product, read this instruction manual thoroughly at first. Do not start the operation until you understand the contents of manual. Also, this document describes only method used for specified purpose with this product. Do not use this product for any purpose or by method other than described here. If you use this product for any purpose or by any method other than described in this manual, you will be held responsible for any necessary safety measures as operator. Additionally, read manual of the equipment you use simultaneously.

2. Application

This product is blocking reagent and dilute solution for western blotting, ELISA, immunostaining of cells and so on.

3. Package

Product name	Volume	Package
EzBlock BSA	200 mL	× 1
Tween 20	10 mL	× 1

4. Components

Product 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 doesn't include notifiable materials exceeding to regulated amount for excluding decided by PRIR Law, Poisonous and Deleterious Substances Control Act and Industrial Safety and Health Law.

5. Storage

- Keep ***EzBlock BSA*** in a refrigerator. If the package is unopened, it is stable until mentioned expiration date.
- Do not mix stock solution of ***EzBlock BSA*** with another blocking reagent etc.

6. Disposal method

- Follow disposal method of each reagent of the organization you belong to.
- Material

  Bottle: High density polyethylene
  Lid: Polypropylene

  Tube (Main body/Lid): Polypropylene

7. Necessary thing other than this product

- Distilled water

8. Precaution 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. Usage

A. In the case of using it as blocking reagent for western blotting

1. **Dilute *EzBlock BSA* 5times with distilled water.** The minimum use amount is 0.65mL/cm². About 50mL is necessary amount for a mini slab gel. 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.

   ※When making working solution of ***EzBlock BSA,*** another blocking reagent can be added.

   ※When working solution of ***EzBlock BSA*** may be diluted more, use ***EzWash*** or TBS-T.
3. Put the above working solution of ***EzBlock BSA*** in the container having capacity that blotting membrane can move in the direction of shaking freely.

4. Immerse blotting membrane in working solution of ***EzBlock BSA*** and shake it at room temperature for 30-60min.

   ※If it takes more than 1 hour for blocking, it may cause over-blocking.

B. In the case of using it as 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 100-300μL 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. Ask the maker about it.

C. In the case of using it as 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 working solution of ***EzBlock BSA*** to the sample so as to cover it enough (about 100μL par 1cm^2).
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.

D. In the case of using it as 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 ***EzWash*** or TBS-T.

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

※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*** □" on the label.
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.

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