

AE-1477 *EzBlock CAS* Instruction Manual

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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 and understand the contents.

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
<i>EzBlock CAS</i>	200 mL	× 1
<i>Tween 20</i>	10 mL	× 1

4. Components

Product name	Main component
<i>EzBlock CAS</i>	Tris (Hydroxymethyl) aminomethane Sodium chloride Casein derived from milk (Hammarsten method) Preservative (This is 5times concentrated stock solution.)
<i>Tween 20</i>	Tween 20 (This is 100times concentrated.)

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

5. Storage

- Keep *EzBlock CAS* in a refrigerator. If the

package is unopened, it is stable until mentioned expiration date.

- Do not mix stock solution of *EzBlock CAS* with another reagent and blocking reagent etc.

6. Disposal method

- Follow disposal method of each reagent of the organization you belong to.
- Materials
 - Bottle (Main body): 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 5times concentrated stock solution of blocking reagent whose main component is casein derived from bovine milk. When you use it, dilute it in accordance with usage.
- 1 bottle of *EzBlock CAS* 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.
- After *Tween 20* is added to blocking reagent, it should be stored in refrigerator and use it within 3days.

9. Usage

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

1. Dilute *EzBlock CAS* 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 CAS* with 39.5mL distilled water.
2. Add 1% amount of *Tween 20* to working solution of *EzBlock CAS*. In the case of the above solution (49.5mL), add 0.5mL *Tween 20* to it.

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

※When working solution of *EzBlock CAS* may be diluted more, use *EzWash* or TBS-T.

- Put the above working solution of *EzBlock CAS* in the container having capacity that blotting membrane can move in the direction of shaking freely.
- Immerse blotting membrane in working solution of *EzBlock CAS* 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

- Dilute *EzBlock CAS* 5times with distilled water.**
When you make 50mL working solution, mix 10mL stock solution of *EzBlock CAS* with 40mL distilled water.
- Add 100-300 μ L of the working solution to each well of 96 well plate.
- 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

- Dilute *EzBlock CAS* 5times with distilled water.**
When you make 50mL working solution, mix 10mL stock solution of *EzBlock CAS* with 40mL distilled water.
- Immerse slide of cell and tissue section in working solution of *EzBlock CAS*. Or, add working solution of *EzBlock CAS* to the sample so as to cover it enough (about 100 μ L par 1cm²).
- 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

- Dilute *EzBlock CAS* 5times with distilled water.**
When you make 50mL working solution, mix 10mL stock solution with 39.5mL distilled water.
- Add 1% amount of *Tween 20* to working solution of *EzBlock CAS*. 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 CAS* may be diluted more, use *EzWash* or TBS-T.

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



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