

## 1. Safety warnings and precautions

Correct operations are necessary for safe use of this product. The complete instructions should be read and fully understood before attempting to use the product. The procedure described in this instruction manual applies only to the use for the intended purpose. Using the product for any purpose other than the intended use or in any manner other than that described in this manual is forbidden. User shall be liable for all safety measures needed for any use other than specified in this manual.

## 2. Introduction

*EzBlock Chemi* is a chemical agent based blocking reagent. It is used for blocking of nonspecific protein bindings to surface of blotting membrane or ELISA plate, and for antibody diluent.

## 3. Package

*EzBlock Chemi* (500mL) ... 1 bottle

## 4. Component

The component of *EzBlock Chemi* is described below.

<p><b><i>EzBlock Chemi</i></b> Tris(hydroxymethyl)aminomethane Synthetic polymer</p>
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*EzBlock Chemi* is a 5-fold concentrated stock solution. It is simply diluted with deionized distilled water before use. *EzBlock Chemi* is a blocking reagent containing nonprotein (Chemical synthetic compound) synthetic polymer. With working solution of *EzBlock Chemi*, approximately 50 sheets of mini-slab gel size membrane can be blocked. *EzBlock Chemi* does not contain sodium azide that inhibits the activity of horseradish peroxidase (HRP). Using *EzBlock Chemi* for the initial testing, antibody concentration should be optimized with the dot blot membrane method, etc.

## 5. Procedure

### A. Using as a blocking reagent for blotting membrane

#### I. Dilute *EzBlock Chemi* 5 times with deionized distilled water.

- The minimum amount for blocking membrane is 0.65 mL/cm<sup>2</sup>, 50 mL is required for mini-slab size.
- *EzBlock Chemi* can be used in combination with other blocking reagents.
- To make more-diluted solution of *EzBlock Chemi* working soln., dilute it with TBS-T or *EzWash* (AE-1480).

#### II. Pour the working solution of *EzBlock Chemi* to the suitable size container.

- In the suitable size (not too wide, not too small) container, the membrane can move freely during the shaking.

#### III. Incubate the blotting membrane in a suitable size container with the *EzBlock Chemi* working solution for 30 minutes.

- Longer incubation (over 60min.) with *EzBlock Chemi* causes over-blocking.

### B. Using as a blocking reagent for ELISA

#### I. Dilute *EzBlock Chemi* 5 times with deionized distilled water.

- Ex) 50mL of the working solution; 10mL of the stock solution diluted with 40mL of deionized distilled water.

#### II. Add the working solution to each well of the titer plate

- For one well of 96 well titer plate, 100 to 300  $\mu$ L of working solution is needed.

#### III. Incubate for 30 to 60 minutes at room temperature.

- After removing the blocking reagent and dried the plate, the blocking titer plate can be kept in a cold storage (2 to 8 °C). The storage period depends on the samples (coating proteins or antibodies).

### C. Using for an antibody diluent

#### I. Dilute *EzBlock Chemi* 5 times with deionized distilled water.

- Ex) 50mL of the working solution; 10mL of the stock solution diluted with 40mL of deionized distilled water.
- The salt and detergent concentration in the working solution should be optimized, according to the antibody to be used.
- Absence of salt or detergent in normal antibody generally causes high background.

## 6. Storage

*EzBlock Chemi* should be stored in a cold storage (2 to 8 °C). Unopened reagent is stable until mentioned expiration date. Do not add other blocking reagents to the *EzBlock Chemi* stock solution. *EzBlock Chemi* is generally shipped at room temperature. Although storage at room temperature for 2 or 3 days does not affect the quality of the product, store it at cold storage (2 to 8 °C) as early as possible after receipt.

## 7. References

Western Blotting, even as performed according to the same protocol, may give different results, depending on technique. If you need our technical tips, contact ATTO Corporation for asking “Tips for Western Blotting”.



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