# *WSE-7240* *EzReprobe* Instruction Manual

June 13th, 2016 Ver.4

## 1. Safety precautions

Before using the product, read this instruction manual thoroughly at first. Do not use it until you understand contents of the manual well. The manual explains methods utilized for specified purposes. Do not use it for any purpose or by any method not described in the manual. If it is used for any purpose or by any method not described in the manual, an operator should take responsibility for all required safety measures and contingencies. Also, read a manual of equipment used with it simultaneously.

## 2. Application purpose

The product is stripping buffer used to peel off antibodies from a membrane after chemiluminescence detection.

## 3. Package

| Name | Volume | Package |
|---|---|---|
| ***EzReprobe*** | 500 mL | 1 bottle |
| ***Enhancer*** | 3 g | 1 small bottle |

## 4. Components

| Name | Major components |
|---|---|
| ***EzReprobe*** | Surfactant, buffer |
| ***Enhancer*** | — (powder) |

The product doesn't include a notifiable material exceeding to regulated amount for exclusion decided by PRTR Law, Industrial Safety and Health Law. If you need (M)SDS of it, please contact our company.

## 5. Preservation method

- Keep ***EzReprobe*** at room temperature (20-30℃). If it is kept at low temperature, the components may be educed from it. However, there is no problem on the quality. Dissolve the components completely at about 30℃ with a warm bath before use. Also, it is stable until the expiration date for use if it is not opened.
- ***EzReprobe*** to which ***Enhancer*** is added should be kept in a refrigerator (2-8℃). Before using, dissolve the components completely at room temperature because it may be educed. Also, it is stable for about 2 weeks in a refrigerator.

## 6. Disposal method

- Follow a disposal method decided by the organization you belong to.
- The product is acid solution(pH3.0). Mix it with used Wash buffer (about 10times the amount of it) or 1M Tris (pH7.5) etc. (1/10 the amount of it) to neutralize. After it is neutralized, dispose it.
- Materials of bottle
  ***EzReprobe*** Main body：Polypropylene
  Lid ：Polypropylene
  ***Enhancer*** Main body：Glass
  Lid：Polyethylene

## 7. Necessary things other than the product

- A blotting membrane after emission detection
- A container for shaker (Tray)
  … Big size enough to move the membrane freely is required when it is shaken.
- Wash buffer（***WSE-7230 EzTBS*** including 0.1% Tween 20, TBS-T or PBS-T etc.)
- Primary antibodies for proteins and labeling secondary antibodies
- A seesaw shaker
- Tweezers

○ For Chemiluminescence detection
(For confirming stripping effect)
- Chemiluminescence substance
  （***WSE-7110 EzWestLumiOne*** etc.）
- Detector（***Ez-Capture*** series, X-ray film etc.）

## 8. Precautions for use

- Keep the product at room temperature (20-30℃). If it is stored at low temperature, the components may be educed from it. However, there is no problem on the quality. Dissolve it completely at about 30℃ with a warm bath before use.
- Keep ***EzReprobe*** to which ***Enhancer*** is added in a refrigerator (2-8℃). It is stable in a refrigerator for 2 weeks. If the components are educed from it, dissolve it completely at room temperature before use.
- The product is acid solution(pH3.0). Mix it with used Wash buffer (about 10times the amount of it) and neutralize it before disposal.
- The product is not available for stripping of blotting membranes detected by coloring of TMB etc.

- Efficiency of stripping is affected by antibody titer and expression level of antigen. To increase the efficiency of stripping for antibodies which are not peeled off easily, extend the reaction time (30min ~ some hours) or deal with it at high temperature (37~50℃). In that time, be careful of denaturation of antigen.

- If the same membrane is stripped some times to detect plural antigen, it begins with antigen of which expression level and titer is low to get good results.

## 9. Usage

Add ***Enhancer***

1. Add 0.6g ***Enhancer*** to 100mL ***EzReprobe,*** and dissolve it.

   ※***EzReprobe*** to which ***Enhancer*** is added is stable in a refrigerator for 2 weeks.

   ※***EzReprobe*** is available without ***Enhancer.*** In the case of antibodies which are not peeled off easily or whose specificity is low, efficiency of stripping is increased and nonspecific adsorption is decreased with ***Enhancer***.

Reaction of ***EzReprobe***

1. After being rinsed with Wash buffer having Tween 20 such as ***EzWash*** etc. , keep a detected membrane in Wash buffer and store it in a refrigerator until stripping.

   ※The preservation term depends on status of blot and stability of antigen.

2. Pour ***EzReprobe*** into a big container enough to move a membrane freely when it is shaken.

   ※The required amount for Mini gel size (85mm x 90mm) is 30mL per a gel.

3. Immerse the membrane in ***EzReprobe*** and shake it at room temperature for 5~15 minutes.

   ※If there is much amount of antigen or antibody titer is high, extend the reaction time or raise the reaction temperature to increase efficiency of stripping.

4. Dispose of ***EzReprobe,*** and rinse the inside of the container with several mL Wash buffer such as ***EzTBS*** (including Tween 20). (Rinse operation)

   ※The product is acid solution (pH3.0). Mix it with used Wash buffer (about 10times the amount of it) to neutralize, when it is disposed of.

5. Repeat a Rinse operation again.

6. Pour 30mL ***EzTBS*** including Tween 20 into the container, and shake it for 3 minutes (Cleaning operation).

   <How to confirm effect of stripping>

   ※You can confirm relic of 2nd labeling antibodies at this stage by reaction with chemiluminescent reagent from a cleaned blotting membrane.

   ※To confirm if primary antibodies are peeled off completely, react a cleaned blotting membrane with 2nd labeling antibodies, and detect relic of primary antibodies with chemiluminescent reagent.

   ※To peel off remaining antibodies completely, react it with ***EzReprobe*** again. Also to increase efficiency of stripping more at that time, extend the reaction time or raise the reaction temperature.

7. Block the membrane with blocking solution such as ***EzBlock BSA.***

8. React new primary and 2nd antibodies sequentially.

**ATTO CORPORATION**
**3-2-2 Motoasakusa,**
**Taito-ku, Tokyo 111-0041, JAPAN**
**Tel +81 3 5827 4863**
**Fax +81 3 5827 6647**
**E-mail: eig@atto.co.jp**
**http://www.atto.co.jp/eng**

Distributed by: