WSE-7140 *EzWestBlue W Instruction Manual*

August 21st, 2022 Ver.1

1. Safety warnings and precautions

Correct operations are necessary Safety warnings and precautions of this product. The complete instructions should be read and fully understood before attempting to use the product.

The Procedure described in the 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 the manual is prohibited.

User shall be liable for all safety measures needed for any use other than specified in the manual.

2. Introduction

EzWestBlueW is a chromogenic substrate for detection of antibodies labeled with horseradish peroxidase (HRP) on western blotting.

This product cannot be used as a chromogenic substrate in ELISA.

3. Package

Name	Volume	quantity
EzWestBlue W	200 mL * One bottle serves for 2000 cm^2 of blotting membrane. (approximately 26 sheets of mini-gel-size membrane)	1 bottle

4. Components

Name	Main component
EzWestBlue W	3, 3', 5, 5' -tetramethylbenzidine · Hydrogen peroxide EzWestBlueW is a clear, color-less or slightly blue solution.

The product doesn't include a notifiable material exceeding to regulated amount for exclusion decided by PRTR Law, Poisonous and Deleterious Substances Control Act, and Industrial Safety and Health Law.

5. Preservation method

- ***EzWestBlue W*** should be stored in a refrigerator at 4-10℃, avoiding light. Unopened reagent is stable until mentioned expiration date.
- ***EzWestBlue W*** turns brown through contact with metal ions. Avoid contaminating ***EzWestBlue W*** with tap water.

6. Disposal method

- Follow the disposal method decided by the organization you belong to.
- Materials of bottles
  (Main body, Lid): Polypropylene

7.Necessary thing other than this product

- Electrophoresis gels
- Blotting membranes, Filter papers
- Electrode reagents
  (Sample buffer, electrode solution, etc.)
- Reagents for Western blotting
  (Transfer buffer, blocking reagent, antibody diluent, washing solution, etc.)
- Primary antibodies and HRP-conjugated secondary antibodies
- A power supply
- A semi dry blotting system
- A SeesawShaker
- Tweezers
- Distilled water
- An imaging system or scanner

8. Precautions for use

- Ready-to-use（preparation is not necessary）
  No reagents need to be added, mixed or diluted.
- Be careful not to contact ***EzWestBlue W*** with metal ions. Otherwise it will turn brown.
- Depending on storage conditions, precipitation may be observed in rare cases, but there is no problem with performance.
- Use antibodies at the concentration recommended by the antibody manufacturer or at a dilution rate of 1/1,000 to 1/10,000 for primary antibody and 1/5,000 to 1/20,000 for secondary antibody.

9. Usage

1. ***EzWestBlue W*** should be warmed to room temperature before use. The required volume of reagents per blotting membrane of a mini size gel (85 mm x 90 mm) is approximately 5 mL.
   ＊The required volume of detection reagent is 100 µL / cm2.

2. After the Western blotting reaction, wash the blotting membrane with a washing buffer such as EzTBS (WSE-7230) containing Tween 20.

   ∗Insufficient washing may result in high background.

   ∗Excessive washing, such as washing with a washing buffer for more than 30 minutes, may weaken the signal.

3. Place the appropriate amount of ***EzWestBlue W*** in a clean tray (larger than the membrane).
   ∗Plastic wrap can be used instead of a tray.

4. Immerse the washed blotting membrane in ***EzWestBlue W***. Make sure that the solution covers the entire blotting membrane.

   ∗ Shaking may disturb signals or reduce sensitivity. Be sure to place the tray still.

5. The reaction time depends on the sample, but the signal begins to be detected immediately after the reaction. Reacting in the dark will reduce the background rise. Generally, react for about 5 to 30 minutes.

   ∗Long reaction time may cause high background.

6. When the desired signal is detected, wash the blotting membrane with distilled water several times to stop the reaction.

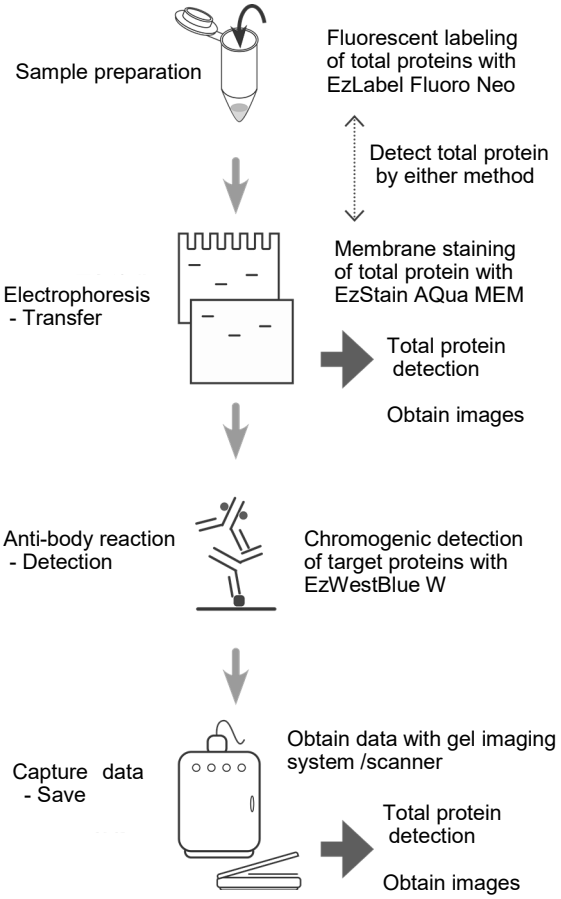
   ∗Acid will cause the signal to turn yellow and become difficult to see, so be sure to use distilled water to stop the reaction.

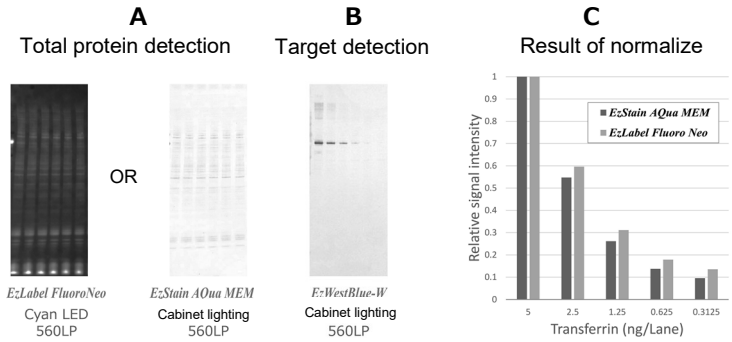
   ∗ When washed with TBS-T, etc., which is used as a washing solution in the antibody reaction of Western blotting, the reaction stops faster than distilled water.

7. After the reaction is stopped, dry the blotting membrane, seal it with Pitatt Clear, etc., and store it in the dark. Since the signal will gradually fade, save the result with an imaging system or scanner.

【When using an imaging system】
Light source: White epi-illumination
(cabinet lighting)
Filter: orange filter (560LPF) or red filter (600LPF)
(can be taken without filters)

10. Application example: Normalization with total protein





Label samples with EzLabelFluoroNeo or stain the blotting membrane with EzStainAQua MEM after transfer, detect the total protein with an imaging system or scanner, and save the images (A).
Then, blocking reaction - antibody reaction is performed, and the signal of the target protein is detected by EzWestBlue W. The detected image is saved with an imaging system or scanner (B).
Using image analysis software such as a CS Analyzer, analyze the images of the total protein (A) and the target protein (B) to obtain the signal intensities of all bands in each lane (A) and the target band (B). The reference band ratio is calculated from the signal intensities of all bands in each lane (A), and the signal intensities of the target band (B) are normalized.

[Reference band ratio] = [Signal intensities of all bands in each lane] ÷[Signal intensities of all bands in reference lane]

[Normalize value] = [Signal intensities of target band] ÷ [Reference ratio]

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