WSE-7140 **EzWest Blue W** Instruction Manual

Nov. 18 , 2025 2nd edition

1. Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time.

2. Purpose of use

This product is a colorimetric substrate for HRP (horseradish peroxidase)-labeled antibodies used in Western blotting. It cannot be used as a colorimetric substrate for ELISA .

3. Product Configuration

Name	Volume	Quantity
EzWestBlue W	200 mL	1 bottle
	(Blotting membrane 2000cm ^{2} minutes) (Approx. 26 mini gels)	

4. Composition

Name	Main component
EzWestBlue W	3,3',5,5'-Tetramethylbenzidine hydrogen peroxide (It is a colorless, transparent to pale blue liquid.)

This Product does not contain any poisonous or deleterious substances under the Poisonous and Deleterious Substances Control Law, or any substances subject to notification that exceed the exemption amounts stipulated under the Industrial Safety and Health Law or the PRTR Law. For details, please download the SDS for this product from the ATTO website (https://www.atto.co.jp/).

5. Storage

- **EzWestBlue W** should be stored in a refrigerator between 2 -10 °C, away from direct sunlight. Unopened, it is stable within the expiration date (approximately one year from the date of manufacture).
- **EzWestBlue W** turns brown when it comes into contact with metal ions. Be careful not to mix it with tap water or other substances.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your institution.
- Bottle material: Body: Polyethylene
  Lid: Polypropylene

7. Items required other than this product

- Electrophoresis gel
- Blotting membrane,Filter paper
- Electrophoresis Reagents
  (sample buffer, electrode solution, etc.)
- Western blotting reagents
  (Transfer buffer, blocking reagent, antibody diluent, washing solution, etc.)
- Primary antibodies and HRP -conjugated secondary antibodies
- Electrophoresis equipment
- Semi-dry blotting device
- Seesaw Shaker
- Tweezers
- Distilled water
- Imaging system, or scanner

8. Precautions for use

- This product is pre-mixed and does not require any reagent addition, mixing, or dilution.
- Please note that **EzWestBlue W** will turn brown when it comes into contact with metal ions.
- Depending on the storage conditions, precipitation may occasionally be observed, but this does not affect performance.
- Use antibodies at the concentration recommended by the antibody manufacturer, or at a dilution of 1/1,000 to 1/10,000 for primary antibodies and 1/5,000 to 1/20,000 for secondary antibodies.

9.How to use

1. Allow **EzWestBlue W** to return to room temperature before use. Approximately 5 mL of reagent is required per mini -size gel (85 mm x 90 mm) blotting membrane.

   * The required amount of detection reagent is 100 µL /cm^2 .

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

   * Insufficient washing may result in high background levels.

   * Excessive washing, such as washing with washing

buffer for more than 30 minutes, may weaken the signal.

3. Place the required amount of **EzWestBlue W** into a clean tray (larger than the membrane).

   * You can use plastic wrap instead of a tray.

4. After washing, immerse the blotting membrane in **EzWestBlue W**. Make sure the solution is evenly distributed over the entire blotting membrane.

   * Shaking at this time may cause the signal to leak or reduce sensitivity. Be sure to leave the sample standing still.

5. The reaction time depends on the sample, but signals will begin to be detected immediately after the reaction. Carrying out the reaction in the dark will help prevent an increase in background. Allow the reaction to proceed for approximately 5 to 30 minutes.

   * If the reaction time is too long, the background may become high.

   Once a signal is detected, the reaction is stopped by washing the blotting membrane several times with distilled water.

   * When using acid, the signal will turn yellow and become difficult to see, so be sure to use distilled water to stop the reaction.

   * Washing with TBS-T, which is used as a washing buffer in the antibody reaction of Western blotting, stops the reaction more quickly than with distilled water.

   After the reaction is stopped, dry the blotting membrane and store it in a dark place, sealed in a Pitatto Clear bag, etc. The signal will gradually fade, so save the image using a camera or scanner.

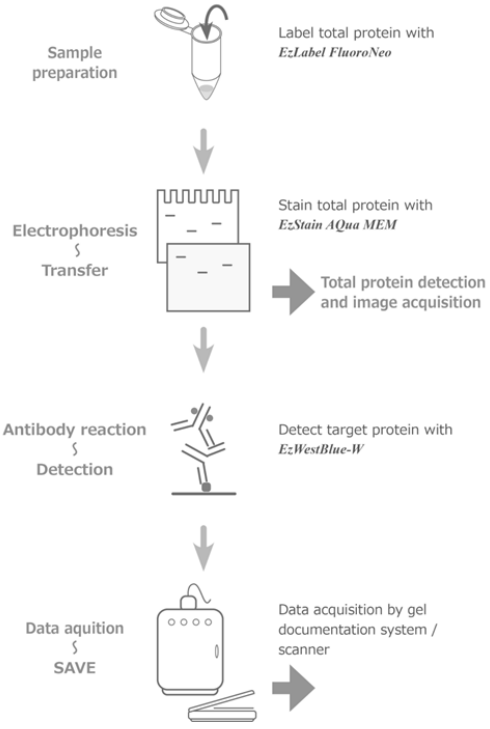
[When taking pictures with an imaging system]

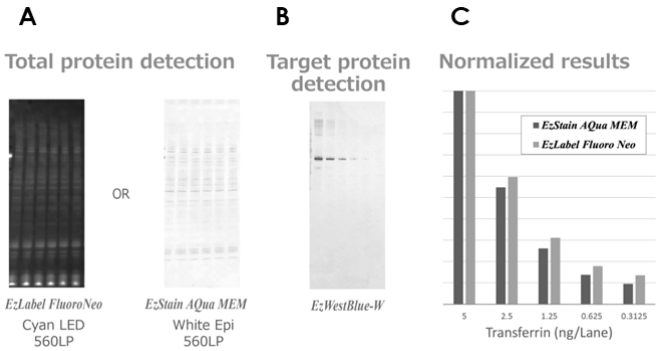
Light source: White reflected light (interior lighting)
Filter: Orange filter (560LPF) Alternatively, a red filter (600LPF) (Photos can be taken without it)

10.Reference materials

Even with the same blotting protocol, slight differences in technique can greatly affect the results. Tips and tricks are also important for obtaining optimal results.
Please read the "Tips for Western Blotting" which can be downloaded from the ATTO website.
https://www.atto.co.jp/

11. Application Examples Normalized by total protein





Label the sample with **EzLabelFluoroNeo**, or stain the blotting membrane after transfer with **EzStainAQua MEM**. Detect total protein using a camera or scanner and save the image (A). Then, perform blocking and antibody reactions, and detect the target protein signal with **EzWestBlue-W**. Save the image (B). Analyze the images of total protein (A) and target protein (B) using image analysis software such as CS Analyzer to obtain the brightness values of all bands in each lane (A) and the brightness values of the target bands (B). Calculate the reference band ratio from the brightness values of all bands in each lane (A), and normalize the brightness value of the target band (B).

*[Reference band ratio (ref. ratio)] = [Brightness values of all bands in each lane] ÷ [Brightness values of all bands in the reference lane]

*[Normalized value] = [Brightness values of target bands] ÷ [Reference ratio]

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