WSE-7120 **EzWestLumi plus** Instruction Manual

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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 luminol-based luminescent substrate used for detection using HRP (horseradish peroxidase)-labeled antibodies in Western blotting analysis.

3. Product Configuration

Name	Volume	Qty
Reagent A Luminol/Enhancer solution Luminol / Enhancer Solution	WSE7120S: 50mL WSE7120L: 250mL	1 pc
Reagent B Peroxide solution Hydrogen peroxide solution	WSE7120S: 50mL WSE7120L: 250mL	1 pc

4.Composition

Name	Main component
Reagent A Luminol/Enhancer solution Luminol / Enhancer Solution	Luminol, enhancer, buffer
Reagent B Peroxide solution Hydrogen peroxide solution	Hydrogen peroxide, stabilizers, buffers

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

EzWestLumi plus should be stored in a dark place and refrigerated (2-10 °C). If unopened, it will be stable until the expiration date.

The working solution of **EzWestLumi plus Reagent A** and **Reagent B** can be stored in a refrigerator and protected from light. Its activity will gradually decrease, so please use it as soon as possible.

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 (PVDF , etc.)
- Filter paper
- Electrophoresis reagents (SDS sample treatment solution, electrode solution, etc.)
- Western blotting reagents (transfer buffer, blocking reagent, antibody diluent, washing solution)
- Primary antibody against the protein of interest and HRP -conjugated secondary antibody
- Electrophoresis equipment
- Semi-dry blotting device
- Seesaw Shaker
- Tweezers
- Chemiluminescence photography device

8. Precautions for Use

- Please store this product in a dark place and refrigerate (2-10 °C). Please note that leaving it at room temperature for a long period of time may cause deterioration.
- This product should be used by mixing **Reagent A** and **Reagent B** in an equal ratio of **1:1.** When collecting the reagent from the bottle, be careful not to mix **Reagent A** and **Reagent B.**
- Use the antibody at the concentration recommended by the antibody manufacturer, or use a dilution of 1/1,000 to 1/10,000 for the primary antibody and 1/50,000 to 1/200,000 for the secondary antibody.

9.How to use

1. Remove the **EzWestLumi plus** from the refrigerator. The required amount of luminescence reagent A and luminescence reagent B per Mini gel - sized (85mm x 90mm) blotting membrane is 2.5mL each.

   * The required amounts of luminescent reagent A and luminescent reagent B are 25 to 100 µL / cm^2 .

2. After reacting with the HRP-labeled antibody, wash the blotting membrane thoroughly with a washing solution such as TBS-T.

   *Insufficient washing may result in high background levels.

3. Prepare the EzWestLumi plus working solution by mixing equal parts of **Reagent A** and **Reagent B**. For

Mini gel -sized blotting membranes, mix 2.5 mL of **Reagent A** and 2.5 mL of **Reagent B**.

4. Add the required amount of **EzWestLumi plus** working solution to a clean tray.

   *You can also use plastic wrap instead of a tray.

5. After washing, immerse the blotting membrane in EzWestLumi plus working solution for a few seconds. Make sure the luminescent reagent is distributed evenly across the entire blotting membrane .

   *Please note that if the blotting membrane is not evenly immersed in the luminescence reagent, it may cause foamy background or unevenness.

6. Place the blotting membrane between clear film or plastic wrap, ensuring no air is trapped.

   *Please note that if air gets between the blotting membrane and the film, it may cause a foamy background or unevenness.

   *When wrapping in plastic wrap, please note that if wrinkles in the wrap form on the surface of the blotting membrane, this may cause wrinkled background or unevenness.

7. Photograph the blotting membrane after reaction with the EzWestLumi plus working solution using a ChemiLumi imaging system.

   *The exposure time varies depending on the concentration of the sample, the titer of the antibody, and the dilution rate. Please consider this for each experiment.

   *Please contact the manufacturer for instructions on how to use the Chemiluminescence imaging device.

10. Reference materials

Even with the same blotting protocol, slight differences in technique can lead to significantly different results. Tips and tricks are also important for obtaining optimal results. Please take a look at the "Western Blotting Tips" that can be downloaded from the ATTO website.
https://www.atto.co.jp/

11. Troubleshooting

Trouble	Solution
Problem 1 : I can't see the band	
Blotting was not performed correctly	When blotting high molecular weight proteins, extending the blotting time and reducing the acrylamide concentration of the running gel can improve the results. When blotting low molecular weight proteins, increasing the amount of methanol added to the blotting reagent by 1.5 to 2 times can improve the results.
The detection system is incorrect	EzWestLumi plus is a luminescent substrate for HRP. Please note that it will not be detectable if a labeled antibody other than HRP is used.
The amount of sample applied is small	If the amount of target protein in the sample is below the detection limit, it will not be detected even if the procedure is performed correctly. Please consider the amount of sample to be applied.
Low/inactive antibody titer	Please confirm that the antibody reacts with the target protein using dot blot analysis etc. Also consider the appropriate dilution rate of the antibody.
Blocking reaction is too strong	Prolonged blocking or high concentration of blocking reagent can cause overblocking, resulting in reduced detection sensitivity. Please consider the type of blocking reagent and blocking time.
Antibody concentration is too low	If the antibody titer is low, it may not be detected due to excessive dilution. Please consider the antibody dilution rate and diluent.
Problem 2 : The band is playing	
Too much sample	Excessive protein contamination can distort the electrophoretic pattern and cause bands to wash out during blotting. As a guideline, apply no more than 5 µg of protein per lane.
Insufficient adhesion between gel and membrane	After stacking the filter paper, gel, and Clear Blot P Plus membrane, press down on the top and roll the special roller to remove excess blotting solution and air bubbles. If there is excess blotting solution between the gel and Clear Blot P Plus membrane, proteins will diffuse into the solution, causing bands to wash out.
Problem 3 : Non-specific bands are detected	
Insufficient cleaning	The strength of the washing solution depends on the detergent concentration, salt concentration, and pH. If there are many non-specific bands, please consider adjusting the detergent concentration and salt concentration.
Degradation or polymerization of the target protein	The sample treatment solution (e.g., EzApply) may have been inactivated. Incompletely reduced samples may form polymers, which may be detected as nonspecific bands. Also, if degradation occurs during protein sample preparation, degradation products may be detected. Please consider re-adjusting the sample.
Non-specific antibody reaction	The antibody epitope may react nonspecifically with other proteins. If the problem persists even after trying different antibody dilutions and blocking conditions, we recommend switching to a different antibody.
Problem 4 : High background noise	
Insufficient blocking	Insufficient blocking reaction can cause high background. Please consider the selection of an appropriate blocking agent, consideration of surfactant and salt concentration, and blocking reaction time.
Insufficient cleaning	To reduce background, it is important to perform a "rinse" step before washing. Be sure to completely remove the reaction solution used in the reaction immediately before washing from the container. Similarly, when replacing the washing solution, be careful not to leave any residue from the previous step. Be sure to perform the washing procedure thoroughly.
The antibody concentration is high.	If the antibody titer is high, even after thorough washing, trace amounts of the antibody may remain and cause background. Please consider the appropriate antibody concentration of the antibody you will be using in advance.
Problem 5 : There is a pattern in the background	
The amount of solution is small	Insufficient volumes of blocking solution, antibody solution, washing solution, etc. may cause increased background and uneven reactions. Please prepare sufficient volumes of the solutions you will be using.
Inappropriate shaking speed	If the shaking speed is inappropriate, triangular waves will appear in the solution. Just below the triangular waves, liquid exchange will be insufficient, causing uneven reactions. Please adjust the shaking speed to prevent triangular waves from appearing.

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