WSE-7110

EzWestLumiOne

operating instructions

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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 with HRP (horseradish peroxidase)-labeled antibodies in Western blotting analysis.

3. Product Configuration

Name	Volume	Quantity
EzWestLumiOne	250mL	1

4. Composition

Name	Main components
EzWestLumiOne	Luminol, enhancers, stabilizers, hydrogen peroxide, buffer solutions

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

- **EzWestLumiOne** should be stored in a dark place and refrigerated (2-10 ℃). If it is unopened, it is stable until the expiration date.

6. Disposal method

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

7. Other items required besides 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 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 in a refrigerator. Please note that leaving it at room temperature for a long period of time may cause deterioration.
- The appropriate dilution for primary antibodies is 1/1,000 to 1/10,000, and for secondary antibodies is 1/50,000 to 1/200,000.

9. How to use

1. Remove EzWestLumiOne from the refrigerator and allow it to warm to room temperature. 5 mL of luminescence reagent is required per minigel size (85 mm x 90 mm) blotting membrane.

   *The required amount of luminescence reagent is 50 to 100µL/cm2 · is.

2. After reacting with the HRP-conjugated antibody, wash the blotting membrane thoroughly with a washing buffer such as **EzTBS-T .**

   *Insufficient washing may result in high background levels.

3. Place the membrane in a clean tray (larger than the membrane) with the desired amount of **EzWestLumiOne**..

   *Use plastic wrap instead of a tray.

4. After washing, immerse the blotting membrane in **EzWestLumiOne** for a few seconds.

   ***If the blotting membrane is not evenly immersed in **EzWestLumiOne**, please note that if the reagent is not applied properly, it may result in a high background or unevenness.

5. Place the blotting membrane in clear film or plastic wrap, making sure there are no air pockets.

   * If air gets between the blotting membrane and the film, bubbles will form. Please be aware that this may result in uneven or streaky backgrounds.

   * If you wrap it in plastic wrap, wrinkles in the wrap may form on the blotting membrane. Please note that if this occurs on the front side, it may cause wrinkled background or unevenness.

6. The blotting membrane after the reaction with **EzWestLumiOne** is photographed using a chemiluminescence imaging system.

*The exposure time depends on the concentration of the sample, the titter of the antibody, and the dilution rate. It depends on the experiment. Please consider it for each experiment.

* For instructions on how to use the chemiluminescence imaging device, please refer to the instruction manual of each manufacturer.

10. Reference materials

Even with the same blotting protocol, slight differences in technique can greatly affect 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. troubleshooting

cause	solution
Problem 1: I can't see the band	
not done properly	When blotting high molecular weight proteins, this can be improved by extending the rototilling time and decreasing the acrylamide concentration of the electrophoresis gel. When blotting low molecular weight proteins, the results can be improved by increasing the amount of methanol added to the blotting reagent by about 1.5 to 2 times.
The detection system is incorrect.	**EzWestLumiOne** is a luminescent substrate for HRP. Please note that it will not be detected if a labeled antibody other than HRP is used.
The amount of sample applied is small	If the amount of the 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.
inactivated antibody titers	Please confirm that the antibody reacts with the target protein by dot blotting etc. Also consider the appropriate dilution rate of the antibody.
Blocking reaction is too strong	Blocking for a long time or using a high concentration of blocking reagent can cause overblocking, resulting in a decrease in detection sensitivity. Consider the type of blocking reagent and blocking time.
Antibody concentration is too low	If the antibody titer is low, it may not be detectable due to over-dilution. Please consider the dilution rate and dilution solution of the antibody.
Problem 2: The band is playing	
Too much sample	If an excessive amount of protein is present, the electrophoretic pattern may become distorted or bands may drift during blotting. As a guideline, apply 5 µg or less of protein per lane.
Insufficient adhesion between gel and membrane	After stacking the filter paper, gel, and Clear Blot P Plus membrane, gently press down on them from above and roll the special roller to remove excess blotting solution and air bubbles. If there is an excess of blotting solution between the gel and Clear Blot P Plus membrane, the protein will diffuse into the solution, causing bands to drift.
Problem 3: Non-specific bands are detected	
Insufficient washing	The strength of the washing solution depends on the detergent concentration, salt concentration and pH. If there are many non-specific bands, please consider the detergent concentration and salt concentration.
Decomposition or polymerization of the target protein	The reducing agent (DTT, etc.) contained in the sample treatment solution (EzApply, etc.) may be inactivated. Incompletely reduced samples may form polymers and be detected as non-specific bands. In addition, if decomposition occurs during protein sample preparation, decomposition products may be detected. Please consider readjusting the sample.
Non-specific reaction of the antibody	The antibody epitope may react non-specifically with other proteins. If the antibody dilution rate and blocking conditions are not improved, we recommend changing to a different antibody.
Problem 4: High background noise	
Insufficient blocking	If the blocking reaction is insufficient, it can cause high background. Please consider the selection of an appropriate blocking agent, the surfactant and salt concentration, and the blocking reaction time.
Insufficient washing	In order to reduce background, it is important to perform a "rinsing" step before the washing operation. 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 washing solution from the previous step. Be careful to perform the washing operation properly.
High antibody concentration.	If the antibody titer is high, even if the washing operation is performed sufficiently, a small amount of 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 amounts of blocking solution, antibody solution, washing solution, etc. can cause an increase in background and uneven reactions. Please prepare sufficient amounts of the solutions you will be using.
The shaking speed is inappropriate	If the shaking speed is inappropriate, triangular waves will occur in the solution. Directly below the triangular waves, the liquid exchange will be insufficient, causing uneven reactions. Please consider the shaking speed so that triangular waves do not occur.

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