WSE-7160

EzStain AQua MEM Instruction Manual

Aug. 21th, 2025 4th 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. Propose of use

This product is a staining and destaining reagent kit for detecting proteins on membranes transferred during Western blotting analysis.

3. Product configuration

Name	volume	Quantity
Wash 2 times concentration solution	500 mL	1bottle
Stain	500 mL	1bottle
de-Stain 2 times concentration solution	500 mL	1bottle
Bleach 2 times concentration solution	500 mL	1bottle

For staining membranes for mini-size gels(90x80mm), The required volume is 20-25 mL (0.2-0.3 mL/cm^2).

4. Composition

This Product does not contain any poisonous or deleterious substances under the Poisonous and Deleterious Substances

Name	Main components
Wash 2 times concentration solution	Buffer
Stain	Coomassie Brilliant Blue, Acidic buffer, stabilizer
de-Stain 2 times concentration solution	Buffer
Bleach 2 times concentration solution	Buffer

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

- Store at room temperature (15-30°C) away from direct sunlight .Please use it unopened and within the expiration date (one year from the date of manufacture). Also, it is stable until the expiration date for use if it is not opened.
- The solution diluted twice with methanol should be stored in a refrigerator (2-10°C). and use it as soon as possible.

6. Disposal method

The "Wash " and "de-Stain" of EzStain AQua MEM are acidic buffer solutions with a pH of 2.0 or less. "Bleach" is a basic buffer solution with a pH of 9.0 or higher. "Stain " is an acidic solution containing pigment with a pH of 2.0 or less. When disposing of the product, please follow the disposal method of your institution.

- Materials of bottle Bottle/Lid : Polypropylene

7. Items required other than the product

- Tray(for dyeing)*
- Beaker
- Shaker
- measuring cylinder
- Methanol

*The tray size allows the blotting membrane to move freely in the shaking direction.

8. Precautions for use

- The following three solutions are double-concentrated solutions. Dilute 2-fold with Methanol. The table shows the approximate amount of solution required for one mini gel.

Name	volume	Methanol
Wash 2 times concentration solution	10 mL	10 mL
de-Stain 2 times concentration solution	20 mL	20 mL
Bleach 2 times concentration solution	10 mL	10 mL

- Destain according to the instructions, and if there is any dye remaining in the background, shake again for about 2 minutes in the "de-Stain" solution.
- Follow the instructions to completely destaining the band. If pigment remains on the band, shake again for approximately 5 minutes in 70% methanol solution, then rinse with pure water.

9. How to use

A. Membrane staining

1. Pour 20 mL of "Wash" solution, diluted 2x with methanol, into the staining tray and completely immerse the membrane immediately after transfer.
2. Shake at room temperature for 2-5 minutes.
   *If the membrane is not hydrophilized, it will cause uneven staining. Shake until the entire membrane is re-hydrophilized.
3. Discard the "Wash" solution and pour 20 mL of "Stain" solution into the tray. Shake well until the membrane is completely immersed.
   *If the membrane is not evenly immersed, it may cause uneven staining. Make sure that the entire membrane is immersed.
4. Once the membrane is completely immersed, shake for 1 minute.
5. Discard the "Stain" solution and pour 20 mL of "de-Stain" solution into the tray.
6. Once the membrane is completely immersed, shake for

2 minutes.

7. Discard the "de-Stain" solution and pour 20 mL of new "de-Stain" solution, then shake for another 2 minutes.
   *If there is still dye remaining in the background after performing the above steps, shake again for approximately 2 minutes with "de-Stain" solution.
8. Remove the membrane from the tray and check the detected protein bands.

Note: Extending the shaking time of the "Stain" solution from 1 minute to 5 minutes or more will result in more intense staining of the bands. When comparing multiple membranes, make sure to standardize the staining time.

B. Confirmation of protein bands

Sandwich the membrane between films such as ATTO "Pitta Clear" and capture the image with a scanner or camera, then save the results. Once the membrane is completely dry, the difference between the background and the bands becomes clearer than when it is wet, allowing for more distinct bands to be detected.

To dry the membrane, use a clean hand towel or similar to absorb moisture from the surface, then air dry for about 5 minutes until there are no uneven areas.

When performing "Total protein Normalization" as described in Section D below, completely dry the membrane before saving the data as an image.

C. Destaining protein bands for antibody reactions

When performing an antibody reaction, completely destaining of the protein bands.

1. Pour 20 mL of "Wash" solution into the tray, completely immerse the membrane, and wash for 5 minutes.
   *If the membrane is dry, shake it until it is completely hydrophilized.
2. Discard the "Wash" solution from the tray and pour in 20 mL of "Bleach" solution.
3. Discard the "Wash" solution from the tray and pour in 20 mL of "Bleach" solution.
4. The protein bands on the membrane are destained.
   *At this point, the entire membrane will turn light blue, but this will not affect the antibody reaction and detection.
   *The dye in Pre-stained markers will not be de-stained.
5. Discard of the "Bleach" solution and rinse with pure water.
   *If any dye remains on the bands after the above operation, shake it in a 70% methanol solution for another 5minutes and rinse with pure water.
6. Start with the blocking procedure, then proceed with the antibody reaction and detection as usual.
   *If not performed blocking procedure immediately, immerse in TBS-T (or PBS-T) and store in the refrigerator.

D. Total Protein Normalization (application example)

1. Using image analysis software such as CS Analyzer, calculate the total protein signal value (Luminance value) from the image data captured in Previous Section B.
2. Calculate the signal value (Luminance value) of all band for each lane. Using the Luminance value of all bands of a given lane as the reference, calculate the ratio between all lane (reference ratio), and use this as a correction coefficient for the variation between each lane.

*[reference ratio] =

$$\frac{\text{[All bands Luminance value of each lane (X)]}}{\text{[All band Luminance value of any lane (N)]}}$$

3. Calculate the Luminance value of the target protein for each lane (Target protein Luminance value) from image data after the antibody reaction.
4. Using the [reference ratio] calculated for each lane in step 2, divide the target protein Luminance value calculated for each lane in step 3 to normalize it.

*[Normalize lane (N)] =

$$\frac{\text{[Luminance value of Target protein in lane (N)]}}{\text{[lane (N)'s reference ratio]}}$$

Confirmation of total protein on membrane after transfer.

Step	Solution	Operate
1.PreTreatment	**Wash**	Shake for 2-5 minutes.
2.Staining	**Stain**	Shake for 1 minute.
3.Destain	**de-Stain**	Shake for 2 minutes twice.

De-staining protein bands for antibody reactions.

Step	Solution	Operate
4.PreTreatment	**Wash**	Shake for 2-5 minutes.
5.Destaining protein bands	**Bleach**	Shake for 5 minute.
6.Rinse with pure water	Pure water	Rinse with Pure water.

10. Reference materials

Even with the same blotting protocol, slight differences in technique can lead to significantly different results .
Please read the "Tips for Western Blotting" which can be downloaded from the ATTO website.

https:// www.atto.co.jp/

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