AE-1460

EzBlot Instruction Manual

August 20,2025 9th 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 blotting buffer used for semi-dry protein blotting .

3. Product configuration

Name	Volume	Quantity
Blotting Buffer A	475 mL	1
Blotting Buffer B	475 mL	2
Blotting Buffer C	475 mL	1
Disposable Tray	-	40

4. Composition

Name	Main component
Blotting Buffer A	2-Amino-2-hydroxymethyl-1,3-propanediol
Blotting Buffer B	2-Amino-2-hydroxymethyl-1,3-propanediol
Blotting Buffer C	2-Amino-2-hydroxymethyl-1,3-propanediol 6-Aminohexanoic Acid

This product does not fall under the GHS classification criteria.For details, please download the SDS for this product from the ATTO website (https://www.atto.co.jp/).

5. Storage

- Store unopened **EzBlot** at room temperature (15 - 30 °C).
- Unopened, it is stable until the expiration date (one year from the date of manufacture)
- After adding methanol , store in the refrigerator (2-10 °C) and use as soon as possible.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your affiliated institution..
- Bottle Material Body and lid : Polypropylene
  Tray Material : polypropylene

7. Items required other than this product

* If you use mini-size gel, the following products are required.

- Methanol
- PVDF membrane (WSE-4051 Clear Blot P Plus membrane)
- 6 filter papers (CB-09A filter paper)
- Semi-dry blotting device
  (WSE-4025 Horize Blot 2M)
  (WSE-4125 Powered Blot 2M)
- Blotting Roller

8. Precautions for use

- This product does not contain methanol. Please add methanol according to the instructions when using. Methanol is a hazardous substance. Use in accordance with the regulations of your institution.

9. How to use

A. Preparation of reagents

This reagent does not contain methanol **.** Add 25 mL of methanol to the bottles of **Blotting Buffer A, B, and C** at the time of use. After adding methanol, check the box on the label . Store in the refrigerator. Add methanol to make a 1x working solution. Ready to use.

B. Hydrophilization of blotting membrane

PVDF membranes require hydrophilization before use . Follow the steps below to hydrophilize the PVDF membrane.

1. Put a few mL of methanol into a container slightly larger than the PVDF membrane, then place the PVDF membrane in it and immerse it for about 10 seconds .
2. Discard the methanol, add approximately 50 mL of **Blotting Buffer B**, and shake for at least 30 minutes. Completely submerge the PVDF membrane in the solution . Be careful as the membrane may float at first . Incomplete hydrophilization can cause uneven blotting efficiency and reduced sensitivity .

C. Blotting

1. Prepare two disposable trays provided and add 50 mL each of **Blotting Buffer A and C**. Immerse filter paper in each tray.

   Blotting Buffer A : 2 sheets

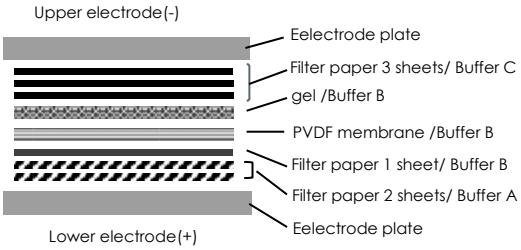
   Blotting Buffer B : 1 sheet
   (Immerse in the same tray as the PVDF membrane in item B-2)

   Blotting Buffer C : 3 sheets

2. After electrophoresis, place the gel in the provided disposable tray containing approximately 50 mL of **Blotting Buffer B**. Gently rinse the gel surface (within a few minutes). Immersing the gel in solution B for a long time will cause a decrease in transfer efficiency.

3. Refer to the diagram below and stack the filter paper, membrane, and gel in that order.

① Add a few mL of **Blotting Buffer A** onto the lower electrode plate to wet it.



② Place two sheets of filter paper soaked in **Blotting Buffer A** on top of the electrode plate.

③ Place one piece of filter paper soaked in **Blotting Buffer B** on top of the filter paper in step ②.

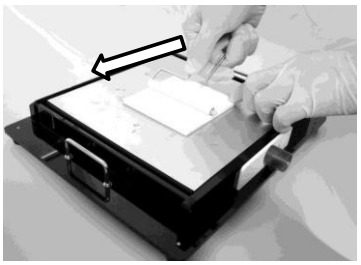
④ Place one PVDF membrane on top.

⑤ Drop a few mL of **Blotting Buffer B** onto the PVDF membrane.

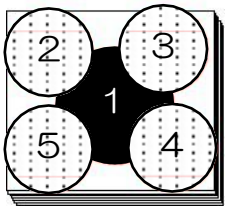
⑥ Carefully place the gel on top of the PVDF membrane, taking care not to trap air bubbles between the gel and the PVDF membrane.

⑦ Place three pieces of filter paper soaked in **blotting buffer C** on top of the gel from step ⑥.

⑧ To improve adhesion between the gel and the PVDF membrane, use the roller attached to the device to push out excess buffer between the gel and the PVDF membrane. Hold down one side of the stacked filter paper with your finger to prevent it from slipping, and press the roller from the pressed area to push out air bubbles and excess blotting buffer.
Do it again from the opposite direction .



If you are not using a roller, press firmly and evenly on several areas from the top with your gloved hands. Insufficient pressure can cause the pattern to blur or result in uneven blotting efficiency.



Press firmly in the center and four areas around the edges.

⑨ Set the electrode plate and apply a constant current of 2 mA/cm per gel area for 30 to 60 minutes..
When using the WSE-4125 Powered Blot 2M, set it to 12V and let it run for 30 to 60 minutes.

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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