AE-1460 EzBlot Instruction Manual

November 11 , 2025 10th 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	capacity	Quantity
Blotting Buffer A	475 mL	1 bottle
Blotting Buffer B	475 mL	2 bottles
Blotting Buffer C	475 mL	1 bottle
Disposable Tray	-	40 sheets

4. composition

name	Main component
Blotting Buffer A	Tris
Blotting Buffer B	Tris
Blotting Buffer C	Tris, 6-aminocaproic acid

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. How to save

- Store unopened **EzBlot** at room temperature (15-30 ° C) . It is stable within the expiration date if unopened (approximately 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 institution.
- Bottle Material Body and lid: Polypropylene
  Tray Material: Polypropylene

7. Items required other than this product

* If you use mini-sized 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 HorizeBlot 2M)
  (WSE-4125 PoweredBlot 2M)
- Blotting Roller

8. Precautions for use

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

9. How to use

A. Reagent Preparation

This reagent does not contain methanol. When using, add 25 mL of methanol to each bottle of Blotting Buffer A, B, or C. After adding methanol, check the box on the label and store in the refrigerator. Adding methanol makes the solution a 1x working solution.

B. Hydrophilization of blotting membrane

PVDF membranes must be hydrophilized in advance. Follow the steps below to hydrophilize PVDF membranes.

1. Pour a few mL of methanol into a container slightly larger than the PVDF membrane, then place the PVDF membrane in it and soak it for about 10 seconds.
2. Discard the methanol, add approximately 50 mL of Blotting Buffer B, and shake for at least 30 minutes. Be sure to completely immerse the PVDF membrane in the solution. Please note that the membrane may float at first. Incomplete hydrophilization may result in uneven blotting efficiency and reduced sensitivity.

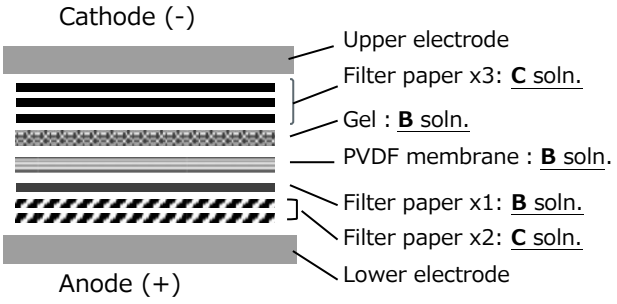
C. Blotting

1. Prepare two disposable trays and place 50 mL each of Blotting Buffer A and C in each tray. 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)
   Blotting Buffer: 3 sheets

2. After electrophoresis, place the gel in the provided disposable tray containing approximately 50 mL of Blotting Buffer B and gently rinse the gel surface (within a few minutes). Immersing the gel in Buffer B for a long period of time may reduce the transfer efficiency.

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



① Blotting Buffer Drop a few mL of solution A onto the lower electrode plate to wet it in advance.

② Blotting on the electrode plate Buffer Place two pieces of filter paper soaked in solution A on top of each other.

③ Blotting Buffer Place one piece of filter paper soaked in solution B on top of the other.

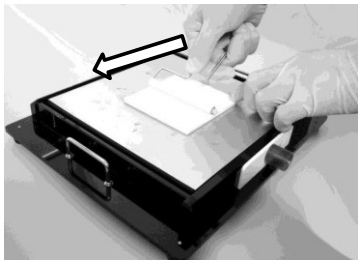
④ Place one PVDF membrane on top.

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

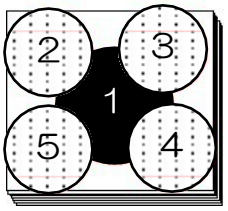
⑥ Carefully place the gel on top of the PVDF membrane, avoiding air bubbles between the gel and the PVDF membrane.

⑦ Blotting Buffer Place three sheets of filter paper soaked in solution C on top of each other.

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



If you are not using a roller, press firmly and evenly on the top of the pattern with your gloved hands. Insufficient pressure can cause the pattern to run 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 2-4 mA/cm^2 per gel area for 30-60 minutes. If using the WSE-4125 PoweredBlot 2M, apply a 12V setting for 60 minutes.

		Condition	Time
Std	c.v.:	12V	60 min
	c.c.:	2 mA/cm^2 (144 mA/gel)	60 min
	c.c.:	4 mA/cm^2 (288 mA/gel)	30 min

The numbers in bracket indicate the conditions for a Mini-size gel (85 x 90 mm).

10. Reference materials

Blotting is the same protocol but with a few minor adjustments. The results can vary greatly depending on the method used. Tips and tricks are also important for obtaining optimal results. Please take a look at the " Tips for Western Blotting" which can be downloaded from the ATTO website. **https:// www.atto.co.jp/**

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