AE- 1465 **EzFastBlot** Instruction Manual

Nov. 11, 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 semi-dry blotting solution used to transfer proteins from a gel to a membrane after electrophoresis.

## 3. Product configuration

| Name | Volume | Quantity |
|---|---|---|
| **EzFastBlot** | 500 mL | 1 |

## 4. Composition

| Name | Main component |
|---|---|
| **EzFastBlot** | Tris(hydroxymethyl)aminomethane<br>6-Aminohexanoic acid<br>(Stock solution 10x the working solution) |

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

- Store unopened **EzFastBlot** at room temperature (15 °C -30 °C).
- It is stable unopened up to the expiration date (one year from the date of manufacture).
- The diluted working solution can also be stored at room temperature, but please use it as soon as possible. It is stable unopened up to the expiration date (one year from the date of manufacture).

## 2. Disposal method

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

## 7. Items required other than this product

* Example: When blotting a Mini-size gel (1 mm thick), the following products are required.

- Blotting membrane
  (WSE-4051 Clear Blot P Plus Membrane, PVDF 85 mm x 90 mm)
- 4 to 6 sheets of filter paper
  (CB-09A filter pape, 85 mm x 90 mm x 0.9 mm)
- Semi-dry blotting device
  (WSE-4025 HorizeBlot 2M)
  (WSE-4045 HorizeBlot 4M)
- power supply
  (WSE-3200 Power Station III )
  (WSE-3500 Power Station HC )
  (WSE-3100 Power Station Ghibli I )

## 8. Precautions for use

- This product is a 10x concentrated stock solution. Dilute with distilled water to a 1/10 concentration.
- Blotting equipment has a maximum current that can be used .

AE-6687 HorizeBlot 2M and AE-6677 HorizeBlot: 1A
AE-6688 HorizeBlot 4 M and AE-6678 HorizeBlot: 1.2A
Using a current greater than this may damage the device.

HorizeBlot 4M units with Lot number 490011 or later can pass a current up to 2.4A. Other manufacturers' blotting devices may also have maximum load currents and voltages, so please check the specifications.

## 9. How to use

A. Dilution of Reagents

Dilute this product with distilled water to a 1/10 concentration.When diluted, it becomes a working solution (blotting buffer).

B. Hydrophilization of blotting membrane

When using a PVDF membrane, hydrophilize the blotting membrane in advance. Follow the steps below to hydrophilize the blotting membrane.

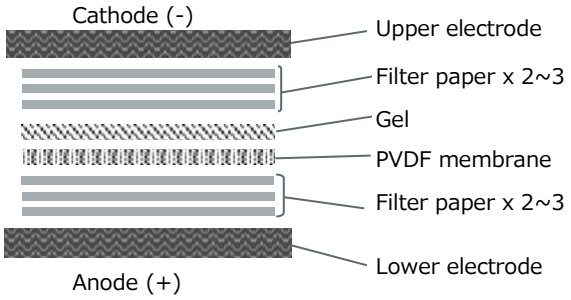
1. Pour a few mL of methanol into a container slightly larger than the PVDF membrane, then place the PVDF membrane in it and soak it for about 10 seconds.
2. 50 mL of blotting buffer in another container, place the membrane removed from the methanol, and shake for 10 to 30 minutes. Be careful as the membrane may float at first, but shake carefully to ensure the PVDF membrane is completely immersed in the solution .

C. Blotting

1. Prepare a container slightly larger than the filter paper, pour approximately 50 mL of blotting buffer into it, and immerse the filter paper in it.

Use 4 or 6 sheets of CB-09A. If using other filter paper of different thicknesses, prepare enough sheets to make the total thickness approximately 4 mm or 6 mm. (See the conditions list below.).

2. After electrophoresis, place the gel in a container containing approximately 50 mL of blotting buffer and gently rinse the surface (within a few minutes) to remove any small gel fragments or SDS bubbles. Do not soak or shake the gel for long time.




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

① Drop a few mL of blotting buffer to the lower electrode plate to wet it in advance.

② Place filter paper soaked in blotting buffer on the lower electrode plate. For WSE-4020, place two sheets, and for AE-6687, place three sheets.

③ Place the blotting membrane on top of the filter paper.

④ Drop a few mL of blotting buffer onto the blotting membrane.

⑤ Place the gel on top of the blotting membrane without trapping any air bubbles between it and the gel.

⑥ Place two or three sheets of filter paper soaked in blotting buffer on top of each other.

⑦ 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. If you do not use a roller, press firmly and evenly from the top in several places with your gloved hands. Insufficient adhesion can cause the pattern to run or uneven blotting efficiency.

⑧ Gently place the upper electrode plate on top of the stack of filter papers and connect the blotting device to the power supply with a lead wire. For the HorizeBlot 2 MR/ 2 M, the lower electrode is the anode (positive pole). Connect the red terminal of the lead wire. Connect the red terminal of the lead wire to the red connector on the power supply.

⑨ For the WSE-4020/4040/4025/4045, apply a constant voltage of 20-25V for 5-20 minutes.
**Hand-cast gel (compact/Mini size gel): 25V**
**Precast gel (compact/Mini size gel): 20V**
The current is approximately 250-500mA per gel.

For the AE-6687/6688, apply a constant current of 6-7 mA/cm$^2$ per gel area for 5-20 minutes.
**Compact gels (60 mm x 60 mm): 250 mA per gel**
**Mini-size gels (85 mm x 90 mm): 450 mA per gel**
The voltage at this time is approximately 25V-40V. Set the power supply to 50V or less.

If you cannot apply the above current, determine the approximate application time by multiplying the current by time. For example, for a Mini-size gel at 288 mA, this is 30 minutes. For conventional conditions, apply a current of 2 mA/cm$^2$ per gel area for 60 minutes.·

If using the PoweredBlot Ace/2M powered blotting system, refer to the system's instruction manual. For the PoweredBlot Mini, the same settings as the EzBlot can be used.

**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/**

| Model | WSE- 4020/4025 | WSE- 4040/4045 | WSE- 4115/4125 | AE- 6687 | AE- 6688 |
|---|---|---|---|---|---|
| Name | HorizeBlot 2M-R/2M | HorizeBlot 4M-R/4M | PoweredBlot Ace/2M | HorizeBlot 2M | HorizeBlot 4M |
| maximum current | - | - | - | 1A | 1.2A |
| Filter papers | 2 sheets x 2 | 2 sheets x 2 | 2 sheets x 2 | 3 sheets x 2 | 3 sheets x 22 |
| Power supply conditions | Constant voltage | Constant voltage | Constant voltage | Constant current | Constant current |
| Fast method (10 min) | 24 V | 24 V | RAPID | 6 mA/cm$^2$ (450mA) | 6 mA/cm$^2$ (450mA) |
| Standard method (60 min) | 12V | 12V | STD | 2 mA/cm$^2$ (144 mA) | 2 mA/cm$^2$ (144 mA) |

*The values in bracket indicate the constant current conditions for one Mini-size gel

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