

SE-7210 EzFastBlot HMW Instruction Manual

Nov. 11, 2025 7th 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 method used to blot proteins from gels onto membranes after electrophoresis.

3. Product Configuration

Name	Volume	Quantity
EzFastBlot HMW	500mL	1 piece

4. Composition

Name	Main component
EzFastBlot HMW	Tris(hydroxymethyl)aminomethane (5x concentrated stock 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

- Unopened **EzFastBlot HMW** can be stored at room temperature (15-30 °C).
- It is stable unopened up to the expiration date (shown on the label) .
- The diluted working solution can also be stored at room temperature (15-30 °C). Please use it as soon as possible.

6. Disposal method

- Dispose of reagents in accordance with the disposal method of your institution .
- Bottle Material Body: High density polyethylene
Lid: Polypropylene

7. Items required other than this product

* Example: When blotting a mini-size polyacrylamide gel (85 x 90 x 1 mm thick), the following products are

required.

- Blotting membrane
(WSE-4051 Clear Blot P Plus Membrane
PVDF 85 mm x 90 mm)
- 6 sheets of filter paper
(CB-09A Filter paper 85 mm x 90 mm x 0.9 mm)
- Semi-dry blotting device
(WSE-4020 HorizeBlot 2M-R)
(WSE-4040 HorizeBlot 4M-R)
(WSE-4025 HorizeBlot 2M)
(WSE-4045 HorizeBlot 4M)
- Power supply
(WSE-3500 Power Station HC)
(WSE-3100 Power Station Ghibli I)

8. Precautions for use

- This product is a 5x stock solution, which should be diluted to 1/5 with distilled water before use .
- Our older blotting equipment has a limit on the maximum current that can be used.

AE-6687 HorizeBlot 2M : 1.0A
AE-6677 HorizeBlot: 1.0A
AE-6688 HorizeBlot 4 M : 1.2A
AE-6678 HorizeBlot: 1.2A

Using a current higher than the above may damage the device. HorizeBlot 4M devices with lot number 490011 or later can draw a current up to 2.4A. Blotting devices from other manufacturers may also have a maximum load current and voltage. Please check the specifications.

9. How to use

A. Dilution of Reagents

Dilute this product with distilled water to a concentration of 1/5 .
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 by following the steps below .

1. Pour a few mL of methanol into a container slightly larger than the PVDF membrane. Place the PVDF membrane in the container and immerse it for about 10 seconds.
2. Add approximately 50 mL of blotting buffer to another container. Place the pretreated membrane removed from the methanol in the container and shake for 5 to 15 minutes. Be careful

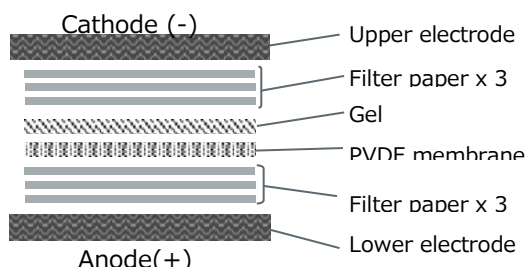
as the membrane may float at first, but shake carefully to ensure that the PVDF membrane is completely immersed in the solution.

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 six sheets of CB-09A. If using filter paper from another manufacturer, cut it to the same size as the gel and prepare enough sheets so that the total thickness is at least approximately 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 a long time.



3. Referring to the diagram above, stack the filter paper, membrane, and gel in the correct order.

- ① Drop a few mL of blotting buffer onto the lower electrode plate to pre-wet the electrode plate.
- ② Place three layers of filter paper soaked in blotting buffer on top of the lower electrode plate.
- 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 three layers of filter paper soaked in blotting

buffer on top of each other.

- ⑦ To improve adhesion between the gel and blotting membrane, use the roller provided with the device to push out excess buffer between the gel and membrane. If not using a roller, press firmly and evenly from above with gloved hands in several places. Insufficient adhesion may cause the pattern to run or uneven blotting efficiency.
- ⑧ Gently lower the upper electrode plate onto the stack of filter paper and connect the blotting device to the power supply with lead wires. For the HorizeBlot 2M-R/2M, 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 15-30 minutes.

Hand-cast gels (compact/mini-size gels): 25V

Precast gels (compact/mini-size gels): 25V

The current required is approximately 250-450mA per gel.

For the AE-6687/6688, apply a constant current of 6-7 mA/cm² per gel area for 15-30 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 required is approximately 25V-40V. Set the power supply voltage to 40V or less.

If you cannot apply the above current, determine the approximate application time by multiplying the current by time. For example, for Mini-size gels, apply 144 mA for 60 minutes.

If you are using the powered blotting devices PoweredBlot ONE or PoweredBlot Ace, please refer to the instruction manual for each device. If you are using the PoweredBlot Mini, you can use it with the same settings as EzBlot.

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/>

Name	HorizeBlot 2M - R / 2M	HorizeBlot 4M - R / 4M	HorizeBlot 2 M	HorizeBlot 4 M
Maximum current	-	-	1 A	1.2 A
Number of filter papers	3 sheets x 2	3 sheets x 2	3 sheets x 2	3 sheets x 2
Power supply conditions	Constant voltage	Constant voltage	constant current	constant current
Fast method (30 min)	24 V	24 V	6 mA/cm ² (450mA)	6 mA/cm ² (450mA)
Standard method (60 min)	12 V	12 V	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



ATTO CORPORATION

3-2-2 Motoasakusa, Taito-ku, Tokyo 111-0041, JAPAN

<https://www.attoeng.com>



Contact