

AE-1465 EzFastBlot Instruction Manual

August 14 , 2025 8th 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	500mL	1

4. Composition

Name	Main component
EzFastBlot	2-Amino-2-hydroxymethyl-1,3-propanediol 6-Aminohexanoic Acid (10x concentrated)

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 **EzFastBlot** at room temperature (15 -30 °C). Unopened, it is stable until the expiration date (one year from the date of manufacture)
The diluted working solution can be stored at room temperature, but please use it 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

7. Items required other than this product

When blotting a mini size gel (1 mm thick)
If so, you will need the following products:

- Blotting membrane
(WSE-4051 Clear Blot P Plus Membrane
PVDF 85mm x 90mm)
- 4-6 sheets of filter paper
(CB-09A Filter paper 85mm x 90mm x 0.8mm)
- Semi-dry blotting device
(WSE-4025 Horize Blot 2 M)
(WSE-4045 Horize Blot 4 M)

- 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 Horize Blot 2 M and AE-6677 Horize Blot: 1A
(Amp)

AE-6688 Horize Blot 4M and AE-6678 Horiz Blot: 1.2A
(Amp)

Using a current greater than this may damage the device.

Horize Blot 4 M lot number 490011 or later The device can deliver current up to 2.4 A.

Other companies' blotting devices may also have limitations on the current and voltage they can use. Please check the specifications.

9. How to use

A. Reagent dilution

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

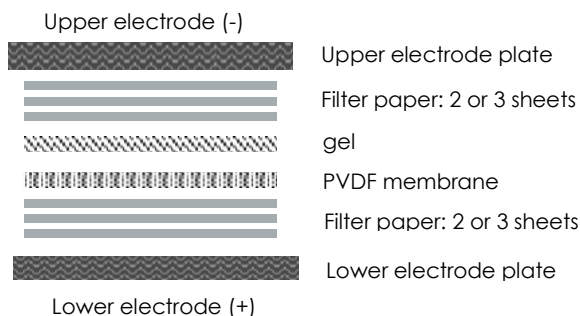
PVDF membrane is hydrophilized by the following steps.

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. 50 mL of blotting buffer to an another container. Place the membrane, which has been hydrophilized with the methanol, in the solution and shake for 10 to 30 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 that is 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 thickness, prepare a number of sheets that will make the total thickness approximately 4 mm or 6 mm (see the conditions table below).
2. After electrophoresis, place the gel in a container containing approximately 50 mL of blotting buffer and gently rinse the surface.
Remove any tiny gel pieces or SDS bubbles.

Do not shake or soak the gel for a 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 onto the lower electrode plate.
- ② Place filter paper soaked in blotting buffer on the lower electrode plate. For WSE-4020, place two pieces, and for AE-6687, place three pieces.
- ③ Place the blotting membrane on top of the filter paper.
- ④ Drop a few mL of blotting buffer onto the PVDF membrane.
- ⑤ Place the gel on top of the PVDF membrane without trapping any air bubbles between membrane and the gel.
- ⑥ Place two or three sheets of filter paper soaked in blotting buffer on top of the gel.
- ⑦ To improve adhesion between the gel and the PVDF 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 blur or uneven blotting efficiency.

⑧ Gently place the upper electrode plate onto the stack of filter papers and connect the blotting device to the power supply with a lead wire. For the Horize Blot 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 WSE-4020/4040/4025/4045, the blotting is carried out at a constant voltage of 20 to 25V for 5-20 minutes.
Hand-cast gels (compact / mini size gels) : **25V**
Precast gels (compact / mini size gels) : **20V**
The current at this time is approximately 250 to 500mA per gel.

The AE-6687/6688, apply a constant current of 6 to 7 mA/cm² per gel area for 5 to 15 minutes.

Compact gel (60mm x 60mm) : **250mA/gel**

Mini size gel (85mm x 90mm) : **450mA/ gel**

The voltage at this time is approximately 25V to 40V .
Set the power supply to 50V or less.

If the above current cannot be applied, the approximate value is calculated by multiplying the current by the time. For example, for mini size gel at 225 mA, the time is 10 to 30 minutes.

When performed under conventional conditions, apply electricity at 2mA/cm² per gel area for 30 to 60 minutes.

If you are using the powered blotting device Powered Blot Ace/2M, please refer to the device's instruction manual. The Powered Blot mini can be used with the same settings as the 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/](https://www.atto.co.jp/)

Model	WSE- 4020/4025	WSE -4040/4045	WSE- 4115/4125	AE- 6687	AE- 6688
name	Horize Blot 2 M - R/ 2 M	Horize Blot 4 M - R/ 4 M	Powered Blot Ace/2M	Horize Blot 2 M	Horize Blot 4 M
maximum current	-	-	-	1 A	1.2 A
Number of filter papers used	2 sheets x 2	2 sheets x 2	2 sheets x 2	3 sheets x 2	3 sheets x 2
Power supply conditions	Constant voltage	Constant voltage	Constant voltage	constant current	constant current
High speed method (5 minutes - 20 minutes)	20-25 V	20-25 V	RAPID	450m A	450m A
Conventional method (20-60 minutes)	12V	12V	STD	144m A	144m A

For one mini-size gel