

1. Safety precautions

Before using the product, read this manual thoroughly at first. Do not start the operation until you understand the contents of manual. This document explains only methods utilized for specified purposes. Do not use the product for any purpose or by any method not described in the manual. If it is used for any purpose or by any method not described in the manual, an operator should take responsibility for all required safety measures and contingencies. Also, read a manual of equipment used with it simultaneously.

2. Application purpose

The product is buffer to transfer proteins from a gel to a membrane by semi-blotting method after electrophoresis.

3. Package

Product name	Volume	Package
EzFastBlot HMW	500mL	1 bottle

4. Components

Name	Major components
EzFastBlot HMW	Tris(Hydroxymethyl) aminomethane (5x stock solution)

The product doesn't include a notifiable material exceeding to regulated amount for exclusion decided by PRTR Law, Poisonous and Deleterious Substances Control Act, and Industrial Safety and Health Law.

5. Preservation method

- Keep unopened **EzFastBlotHMW** at room temperature.
- It is stable until the expiration date for use (denoted on the label) if it is not opened.
- The working solution is also storable at room temperature. But, use it up as soon as possible.

6. Disposal method

- Follow a disposal method established by the organization you belong to.
- Materials of bottle
 - Main body : High density polyethylene
 - Lid : Polypropylene

7. Necessary things other than the product

※e.g., the following products are required for blotting of a polyacrylamide gel of Mini size (85 x 90 x 1 mm thick).

- A membrane
(WSE-4051 P plus membranes PVDF 85 mm x 90 mm)
- 6 filter papers
(CB-09A Filter papers 85 mm x 90 mm x 0.9 mm)
- A semi dry blotting system
(WSE-4020 HorizeBLOT 2M-R)
(WSE-4040 HorizeBLOT 4M-R)
(WSE-4025 HorizeBLOT 2M)
(WSE-4045 HorizeBLOT 4M)
- A power supply
(WSE-3100 powerStation Ghibli I)
(WSE-3500 powerStation HC)

8. Precautions for use

- The product is 5x stock solution. Dilute it to the density of 1/5 with distilled water, when it is used.
- The blotting devices of our old model limit max electric current (Ampere) as follows.
AE-6687 HorizeBLOT 2M : 1.0A
AE-6677 HorizeBLOT : 1.0A
AE-6688 HorizeBLOT 4M : 1.2A
AE-6678 HorizeBLOT : 1.2A
If you use higher current than the above, the device may be broken.
HorizeBLOT 4M whose lot number is after 490011 can provide electric current till 2.4A.
Also, a device of other company may limit max current and voltage.
Confirm the specification before an operation.

9. Usage

A. Dilute reagent

To make the working solution (blotting buffer), dilute the product to the density of 1/5 with distilled water.

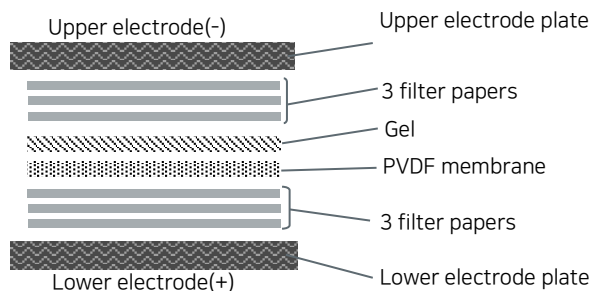
B. Make a blotting membrane hydrophilic

If a PVDF membrane is used for blotting, make it hydrophilic by the following method in advance.

1. Pour several mL methanol into a container bigger than a PVDF membrane. Immerse it in the methanol for about 10 seconds.
2. Pour about 50 mL blotting buffer into another container. Take the membrane out from methanol and put it in the blotting buffer. Shake it for 5~15 minutes. It tends to float at the beginning. Shake it to immerse in the solution completely.

C. Blotting

1. Prepare 6 filter papers (CB-09A). If you use filter papers of other company, cut it to the same size as a gel, and prepare the number of it so as to be more than 6 mm thick in total. Pour about 50 mL blotting buffer into a container bigger than it, and immerse it in the solution. **(Refer to conditions on the below.)**
2. After electrophoresis, put a gel in about 50 mL blotting buffer, and rinse the surface lightly **(within several minutes)** to get rid of small bits of gel and bubbles of SDS. Do not immerse or shake it for a long time.



3. Pile filter papers, the membrane and the gel with referring to the above figure.
 - ① Drop several mL blotting buffer into the lower electrode plate to get wet in advance.
 - ② Put 3 filter papers immersed in blotting buffer on the electrode plate.
 - ③ Put the membrane on the filter papers.
 - ④ Drop several mL blotting buffer into the membrane.
 - ⑤ Put the gel on the membrane without inserting air bubbles between them.

- ⑥ Put 3 filter papers immersed in blotting buffer on the gel.
- ⑦ To enhance adhesion of the gel and membrane, push out unnecessary buffer with a roller attached to the device. Or, push some places of the pile strongly and evenly from above by hands wearing gloves. If the adhesion is insufficient, smiling or uneven blotting efficiency may occur.
- ⑧ Set the upper electrode plate on the filter papers calmly. Connect the device and a power supply with lead wires. The lower electrode of HorizeBLOT 2M-R/2M is anode (+). Connect the red terminal of lead wires to the red connector of power supply.
- ⑨ Apply 25V of constant voltage for 15~30 minutes in the case of WSE-4020/4040/4025/4045.
 - Hand-made gel (Compact/Mini size gel): 25V**
 - Precast gel (Compact/Mini size gel): 25V**
 Set current value as about 250~450 mA per a gel at that time.

Apply 6~7 mA of constant current per 1 cm² for 15~30 minutes in the case of AE-6687/6688.

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

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

Set voltage value as 25~40V at that time. The voltage should be set as 40V or less.

If current can not be set as the above, adjust the time to apply it. For example, if 225 mA of electric current flows in a Mini size gel, the time is 30~60 minutes.

If WSE-4110 poweredBLOT-ONE which is built in power supply unit is used with the product, read the instruction manual of the equipment thoroughly.

Model	WSE-4020/4025	WSE-4040/4045	AE-6687	AE-6688
Name	HorizeBLOT 2M-R/2M	HorizeBLOT 4M-R/4M	HorizeBLOT 2M	HorizeBLOT 4M
Max current	-	-	1A	1.2A
The number of filter papers	3 papers x 2	3 papers x 2	3 papers x 2	3 papers x 2
Condition	Constant voltage	Constant voltage	Constant current	Constant current
Rapid method (15-30min)	25V	25V	450mA	450mA
Conventional method (30-60min)	12V	12V	144mA	144mA

*For 1 Mini size gel



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