AE-1465 ***EzFastBlot*** instruction manual

1. Safety warnings and precautions

Correct operations are necessary for safe use of this product. The complete instructions should be read and fully understood before attempting to use the product. The procedure described in the instruction manual applies only to the use for the intended purpose. Using the product for any purpose other than the intended use or in any manner other than that described in the manual is forbidden. User shall be liable for all safety measures needed for any use other than specified in the manual.

2. Introduction

EzFastBlot is a alcohol-free blotting buffer for semi-dry electroblotting from polyacrylamide gels to the membrane. In high-current conditions (ex. 450mA, 10min.), ***EzFastBlot*** is able to transfer the samples faster than the general blotting buffer. The preparation is easy to dilute it five times with DDW.

3. Package

Blotting Buffer（500mL, 10x stock solution）･･･ 1 bottle

4. Component

The concentration described below, refers to the final concentration after DDW is added according to "Procedure".

Blotting Buffer

· 600 mM Tris

· 50 mM 6-aminocapronic acid

5. Procedure (for a mini-gel blotting)

Additional Materials required

· DDW

· Blotting membrane (AE-6665P ClearBlot membrane P)

· 6 sheets of filter paper (CB-09A Absorbent paper)

· Semi-dry blotting apparatus (WSE-4020/4040, HorizBlot series)

Preparation of reagents

The bottle contains 10x stock solution. Preparing 1x working solution, DDW and ***EzFastBlot*** (10x stock) are mixed in the ratio four to one. For example, 250 mL of 1x working solution is prepared mixing 225mL of DDW and 25mL of ***EzFastBlot*** (10x stock).

Hydrophilization of blotting membrane(Preparation of blotting membrane)

Using a PVDF membrane, the blotting membrane should be hydrophilized prior to use. The procedure for hydrophilizing a blotting membrane is described below.

1. Pour a several milliliters of methanol in a container (the size larger than the PVDF membrane), and soak the PVDF membrane (ex. the membrane size for mini-gel is 85 mm x 90 mm) for approximately 10 seconds.

2. Discard methanol and pour approximately 50 mL of 1x working solution, and then incubate the PVDF membrane for over 30 minutes at least. Make sure that the PVDF membrane is completely soaked and remained under the 1x working solution. Incomplete hydrophilization may cause non-uniformity or low sensitivity of blotting.

Blotting

1. Pour 50 mL of 1x working solution in a tray and soak the filter papers. Using CB-09A, 6 sheets are soaked. If other filter paper use, some pieces are soaked that thickness of stacked them are about 6 mm.

2. After electrophoresis, soak the gel in a tray containing approximately 50 mL of 1x working solution. Gently rinse the surface of the gel to remove air bubbles prevents the transfer. Prior to blotting, all of air bubbles should be removed. Since excessive soak may result in reduced blotting efficiency or diffuse bands, the soak of gel in 1x working solution should not exceed several minutes.

3. Stack the gel, the membrane and the filter papers, as follows.

1) Wet the electrode plate by dropping 1x working solution.

2) Stack some filter papers that thickness of piled up them are about 3 mm (ex. three sheets of CB-09A).

3) Place the pre-wetted blotting membrane on top of the wetted filter paper.

4) Drop 1x working solution and stack the gel on the blotting membrane, taking care to avoid air bubbles.
5) Stack some filter papers on the gel. The thicknesses of them are same as 2).
6) Push out excess of buffer and press down the gel strongly and uniformly with gloved hands. It enhances the contact between the gel and the membrane.
7) Connect the electrode plates and begins the transfer. Fast-blotting condition, the current is 6 ~ 7 mA/cm^2 for 10 to 15 minutes. In this condition, the voltage of power supply builds up to 30 ~ 40 V. The power supply should be set the voltage does not exceed over 50 V.

ex. Membrane of mini-gel size (85 mm x 90 mm) : 450mA
compact size (60 mm x 60 mm) : 250mA

Normal-blotting condition, the current is 2 mA/cm^2 for ~ 60 minutes.

6. Storage

· ***EzFastBlot*** should be stored at room temperature. Do not keep at cold storage because the component is precipitated. Unopened reagent is stable until mentioned expiration date.

7. References

Blotting, even as performed according to the same protocol, may give greatly different results, depending on technique. For best result, you can read “Tips for Western Blotting” at the homepage of ATTO Corporation (http://www.atto.co.jp)

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