AE-1460 EzBlot

1. Safety warnings and precautions

Correct operations are necessary Safety warnings and precautions 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 forbided.
User shall be liable for all safety measures needed for any use other than specified in the manual.

2. Introduction

EzBlot is a transfer buffer for semi-dry electroblotting from polyacrylamide gels.

3. Package

Blotting Buffer A(475mL) ･･･ 1bottle
Blotting Buffer B (475mL) ･･･ 2bottles
Blotting Buffer C (475mL) ･･･ 1bottle
Disposable tray ･･･ 40pcs

4. Component

EzBlot contains no methanol. Add methanol according to “Procedure,” before use.
The concentration of each solution, described below, refers to the final concentration after methanol is added.

Blotting Buffer A (475mL)
· 300 mM Tris
Blotting Buffer B (475mL)
· 25 mM Tris
Blotting Buffer C (475mL)
· 25 mM Tris
· 40 mM 6-aminocapronic acid

5. Procedure (for a mini-gel blotting)

Additional Materials required

· Methanol
· Blotting membrane (AE-6665P membrane)
· 6 sheets of filter paper (CB-09A filter paper)
· Semi-dry blotting apparatus (AE-6687/6688, HorizBlot)

Preparation of reagents

The reagents incorporated in EzBlot contain no methanol. Add each 25 mL of methanol to *Blotting Buffer A*, *B* and *C*, and then mark the checkboxes. The resultant solutions become the respective 1×working solutions, and can be used without further dilution.

Hydrophilization of blotting membrane(Preparation of blotting membrane)

In case of 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 (a size larger than the PVDF membrane), and soak the PVDF membrane (85 mm x 90 mm) for approximately 10 seconds.
2. Discard methanol and pour approximately 50 mL of *Blotting Buffer* B, and then incubate the PVDF membrane for over 30 minutes at least. Make sure that the PVDF membrane is completely remained under the solution. Incomplete hydrophilization may cause nonuniformity or low sensitivity of blotting.

Blotting

1. After finished electrophoresis , soak the gel in a disposable tray included in EzBlot package, containing approximately 50 mL of *Blotting Buffer B*. Gently rinse the surface of the gel to remove air bubbles: Removal of air bubbles before blotting prevents the occurrence of air bubbles during blotting. Since excessive soak may result in reduced blotting efficiency or diffuse bands, the soak of gel in the buffer should not exceed several minutes.

2. Pour 50 mL of *Blotting Buffer A* in a disposable tray included in EzBlot package. Similarly, pour 50 mL *Blotting Buffer C* in another disposable tray. Immerse 2 sheets and 3 sheets of filter paper, respectively, in *Blotting Buffer A* and *C*.

Blotting Buffer A 2 sheets
Blotting Buffer C 3 sheets

Immerse one sheet of filter paper in the tray containing the PVDF membrane.

Blotting Buffer B 1 sheet

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

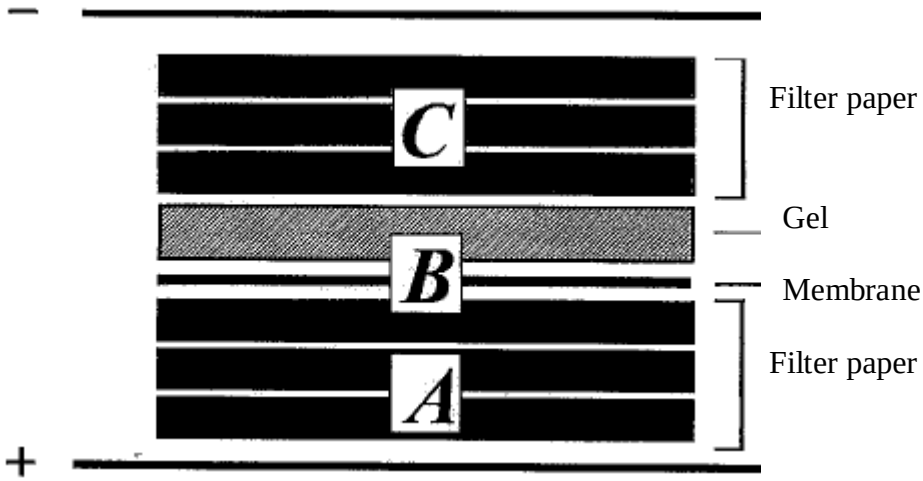
1) Wet the electrode plate by dropping *Blotting Buffer A* (several milliliters) in the tray.
2) Stack the 2 sheets of filter paper pre-wet in *Blotting Buffer A* on the electrode plate.
3) Place the 1 sheet of filter paper pre-wet in *Blotting Buffer B* on top of the filter paper of 3).
4) Place the pre-wetted blotting membrane on top of the wetted filter paper.
5) Drop *Blotting Buffer B* (several milliliter) from the tray on the blotting membrane.
6) Stack the gel on the blotting membrane, taking care to avoid air bubbles.
7) Place the 3 sheets of paper filter pre-wet in Blotting Buffer C on the top of the gel.
8) Push out excess of buffer and press down the gel strongly and uniformly with gloved hands to enhance the contact between the gel and the membrane.
9) Connect the electrode plates and begins the transfer at 2 mA/cm^2 for 30 to 60 minutes.

6. Storage

· EzBlot should be stored at room temperature, avoiding direct light. Unopened reagent is stable until mentioned expiration date.
· The reagents mixed with methanol should be stored in a refrigerator and use these as early as possible.

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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