Transfer Efficiency of E-Blotter

INTRODUCTION

Western Blotting (WB), also known as protein blotting or immunoblotting, allows the transfer of proteins from a sodium dodecyl sulfate (SDS) polyacrylamide gel to an adsorbent membrane, made from e.g. Polyvinylidene Fluoride (PVDF) or nitrocellulose. Three main processes for transferring proteins from a gel to an adsorbed membrane have been described thus far; (1) simple diffusion (Renart et al., 1979; Kurien and Scofield, 1997); (2) vacuum-assisted solvent flow (Peferoen et al., 1982); and (3) ‘‘Western’’ blotting or electrophoretic elution (Towbin et al., 1979). Electrophoretic elution, or electrotransfer, is today by far the most commonly used transfer method. Electrophoretic transfer can be utilized as either wet or semi-dry systems. In wet transfer systems, the gel/membrane stack is completely immersed in an electrode wired buffer tank. Semi-dry transfer systems utilize less buffer. The buffer tank is replaced by two electrode plates. A stack of gel/membrane and buffer-soaked filter papers is placed in between the electrode plates, thus creating a closed circuit.

Since factors such as polarity and size of proteins influence transfer ability, voltage, current and transfer times required for efficient transfer of proteins from a gel to a membrane may vary greatly. Wet transfer, which requires a larger buffer volume, typically runs at constant voltage and is an effective but slow technique, whereas the less buffer-consuming semi-dry system usually runs at constant current and is a less effective but far quicker method.

An important aspect of transfer is the transfer buffer. The buffer should provide an electrical continuity between the electrodes and a chemical environment that maintains the solubility of the proteins without preventing the adsorption of the proteins to the membrane during transfer. Traditional transfer buffers consist of a Tris-Glycine buffer supplemented with methanol. Most buffers undergo Joule heating during transfer. Increased heat may have an effect on the transfer of proteins.

For this reason, many wet transfer systems are equipped with built-in cooling coils. Alternatively, blotting tanks can be placed in a cold room. The design of Wealtec's E-blotter system eliminates the need for ice-packs and external cooling. All that is required is for the transfer buffer to be pre-chilled.

The purpose of this application note is to demonstrate the transfer-efficacy with Wealtec's wet blotting system, E-Blotter.

MATERIALS AND METHODS

Sample preparation

- K562 cell lysate (Emo Biomedicine Corp.)
- 1 mg/ml BSA; 0.05 g BSA (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.) dissolved in 50 ml ddH_2O, aliquoted to 1 ml in eppendorf tubes and freezed in -20°C
- Pre-stained protein marker (Bio-Rad, Hercules, CA, U.S.A.)
- Gel-solutions (Bio-Rad)
- V-GES electrophoresis system (Wealtec, Taipei, Taiwan)
- E-blotter wet blotting system (Wealtec)

Protein electrophoresis

BSA samples and serial dilutions of K562 cell lysate mixed with 4X protein loading dye and a pre-stained marker were loaded onto 12 % SDS-PAGE (0.75 mm) gels. Electrophoresis was performed under following condition; 60 minutes at 90 V, and then 60 minutes at 130 V. After the electrophoresis, the gel was transferred to a PVDF membrane using E-Blotter (Wealtec), at constant 100 V for 1 hr. Transfer was performed with or without ice packs.

Coomassie Blue Staining

After electrophoretic transfer, the PVDF membrane and the gel were stained with Coomassie Blue Staining Buffer; 0.1 % Coomassie Blue R-250 (Bio-Rad) in Water/Methanol/Acetic acid (45:45:10). Thereafter, gels were destained in destaining Buffer; Water/Methanol/Acetic acid (45:45:10).
Images of gels and membranes were captured using by Dolphin-Doc plus image system (Wealtec).

RESULT

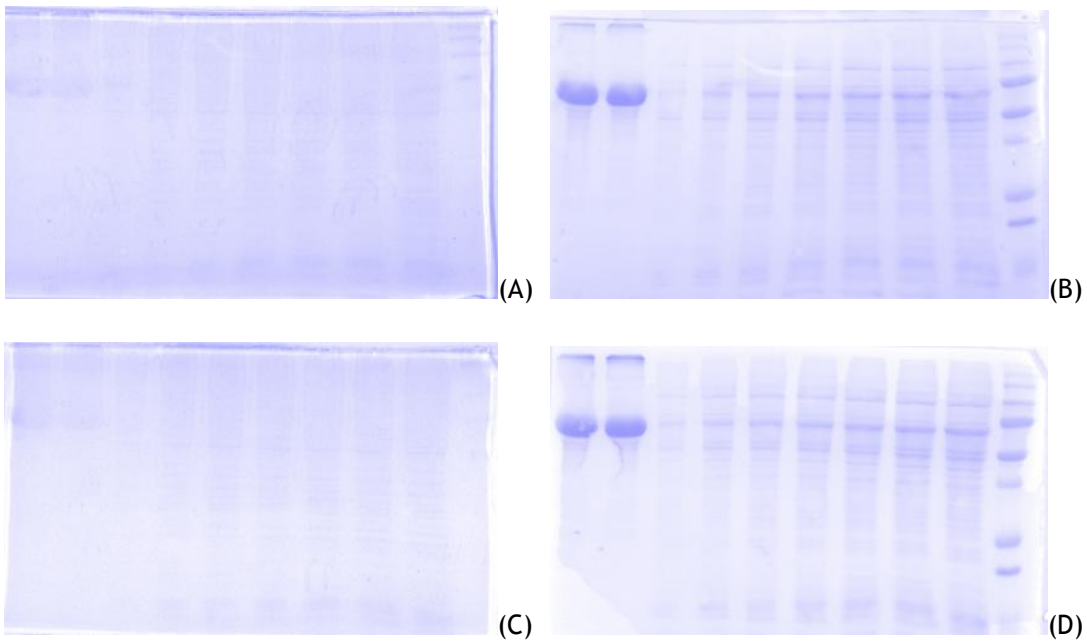

Figure 1. *Transfer efficiency of E-Blotter with ice pack. (A) Gel after transfer for 30 min. (B) PVDF after transfer for 30 min. (C) Gel after transfer for 1 hr. (D) PVDF after transfer for 1 hr.*

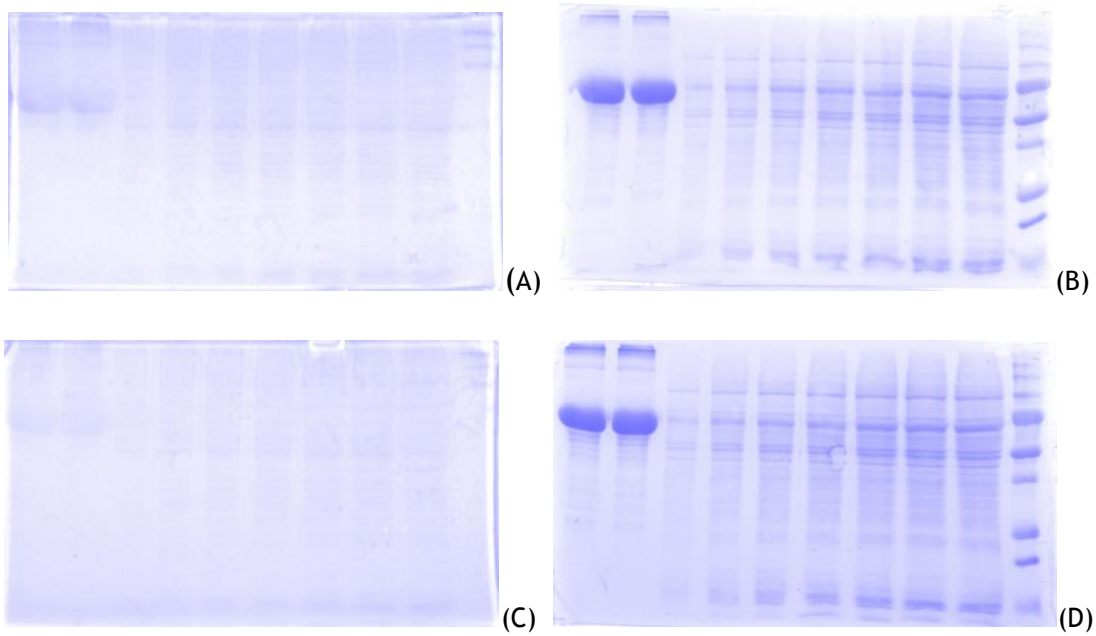

Figure 2. *Transfer efficiency of E-Blotter without ice pack. (A) Gel after transfer for 30 min. (B) PVDF after transfer for 30 min. (C) Gel after transfer for 1 hr. (D) PVDF after transfer for 1 hr.*

DISCUSSION

In this application note, we show the efficacy of the E-Blotter with no additional cooling after only 30 min transfer of protein from a K562 cell lysate.

A comparison between transfer results of E-blotter with and without ice-pack (shown in Figure 1 and 2) reveals that additional cooling does not increase efficacy of protein transfer worth mentioning. The lack of extra protein transfer when adding transfer time to the original 30 minutes further demonstrates the efficacy of the E-blotter wet system. The transfer efficiency is virtually the same when transferring for 30 min and 1 hr (Figure 1A and 1B; Figure 2A and 2B).

The K562 cell lysate contains proteins of large as well as small molecular weight and also proteins of different polarity. The transfer results of separated proteins from the cell lysates are equally good regardless if extra cooling is used or not. However, it should be noted that there is a large variance in requirements for the transfer of different proteins. Proteins of extremely large molecular weight might require increased voltage or increased transfer times, and these are factors that will increase the heat generated during transfer. In these cases additional cooling, e.g. ice packs can be used in order to reduce the risk of heat induced impact on transfer results.

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