Evaluate The Influence of Transfer Time on Semi-Dry Blotting Results

INTRODUCTION

The transfer of electrophoresed proteins from polyacrylamide gel electrophoresis to a membrane such as polyvinylidene difluoride (PVDF), nitrocellulose (NC), cellulose acetate (CA), polyethersulfone (PES), or nylon is defined as Western blotting transfer. Besides the standard Western blot, there are now a lot of techniques for transferring protein from gel to membrane, including capillary transfer, heat-accelerated convectional transfer, vacuum blotting, and electroblotting (electrotransfer). Electroblotting transfers protein to the membrane by using an electric field as a driving factor. With its advantages of being fast, efficient, and yielding high-resolution protein patterns, electroblotting has become the most frequently and widely used transfer technique. Semi-dry blotting is a kind of electroblotting that offers several benefits, including the use of significantly less buffer, the ability to set up the gel-membrane sandwich quickly, and the use of multiple transfer buffers.

Yrdimes is a semi-dry transfer device from Wealtec that is built with a bi-directional force design that utilizes eight pieces of springs to produce the most uniform force during blotting, bringing the electrode into contact at all working areas to provide great blotting results. A Yrdimes may transfer up to four mini gels (10x8 cm) simultaneously, and the system's ELITE power supply offers ultra-stable output during the transfer. The transfer time points for a small-molecule-weight protein marker (35-180 kDa) were examined in this paper using Bjerrum and Schafer-Nielsen transfer buffers. The goal of the research is to determine the minimum time to achieve a good transfer signal by using Yrdimes.

The **KETA GLX** gel documentation system was used to capture the results. The KETA GLX has a 16-bit B/W 5.0 M pixel camera, a computer-controlled lens, a five-position motorized filter wheel with a high transparency amber filter, and a 2X close-up lens, making it suitable for professional picture capturing.

MATERIAL

- 30% acrylamide (Sigma-Aldrich)
- Tris-HCl 1.5M pH 8.8
- Tris-HCl 0.5M pH 6.8
- SDS 10% (Sigma-Aldrich)
- APS 10% (Sigma-Aldrich)

- TEMED (Sigma-Aldrich)
- Prestained protein marker 10-180 kDa (EBL)
- 1X sample buffer
- 1X running buffer
- Bjerrum and Schafer-Nielsen transfer buffers
- Coomassie brilliant blue (Nacalai Tesque)
- 7 x 8 cm filter paper
- 7.5 x 4.5 cm Immobilon-P PVDF Membrane 0.45 µm pore size (Sigma Aldrich, US)
- Instruments: Yrdimes Semi-Dry system, V-GES, ELITE 300U and HC 2.5, and KETA GLX (Wealtec)

METHODS

Polyacrylamide gel electrophoresis

- Prepare the resolving gel with 3.8 mL DDW, 3.4 mL 30% acrylamide, 2.6 mL Tris-HCl 1.5M pH 8.8, 100 µL SDS 10%, 100 µL APS 10%, and 10 µL TEMED.
- Prepare the stacking gel with 5.86 mL DDW, 1.34 mL 30% acrylamide, 2.6 mL Tris-HCl 0.5M pH 6.8, 100 µL SDS 10%, 100 µL APS 10%, and 10 µL TEMED.
- Prepare the prestain protein marker with 67.5 µL marker and 202.5 µL 1X sample buffer. Load 10 µL marker in each well.
- Run the gels at 60V for 40 min and then 120 V for 70 min with 1X running buffer. After the electrophoresis has finished, collect the gel.

Semi-dry blotting

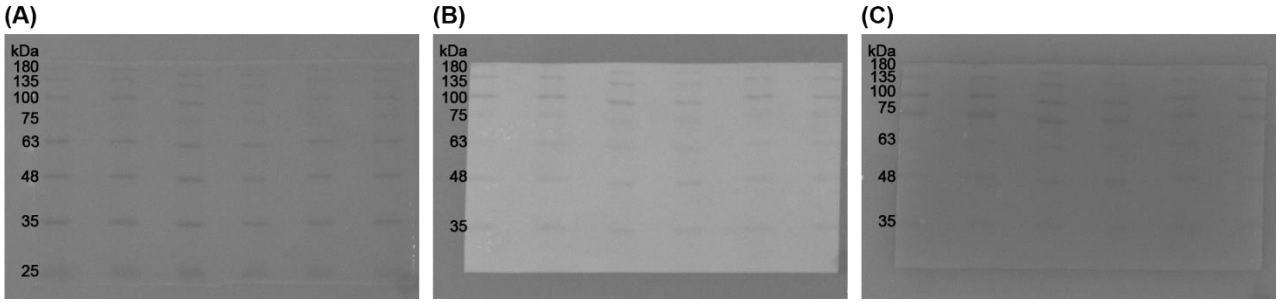
- Activate PVDF with methanol, DDW, and transfer buffer.
- Soak filter papers in transfer buffer before using.
- Assemble the transfer sandwich as follows: (1) anode, (2) three pieces of filter papers, (3) membrane, (4) gel, (5) three pieces of filter papers, and (6) cathode. Use a roller to remove bubbles.
- Pour additional transfer buffer on top of the sandwich.
- Set the transfer current to 180 mA for 41/42/43 min.

CBB staining

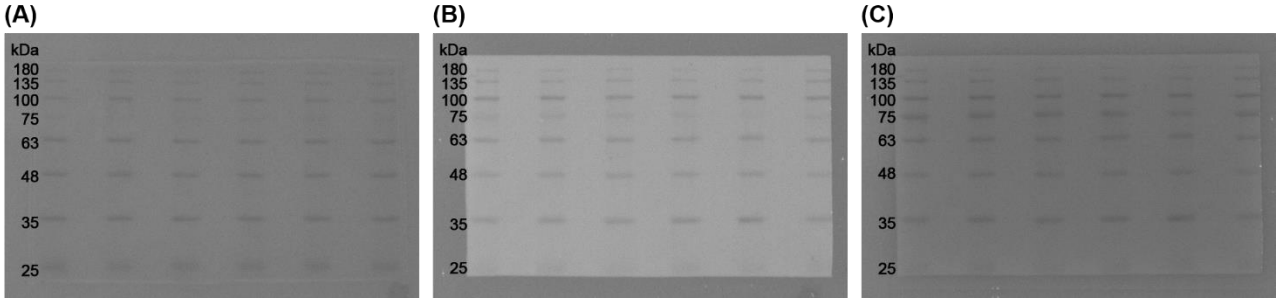
- Stain the gel and membrane with CBB for an hour.

RESULT

- Transfer time: 41 minutes



- Transfer time: 42 minutes



- Transfer time: 43 minutes

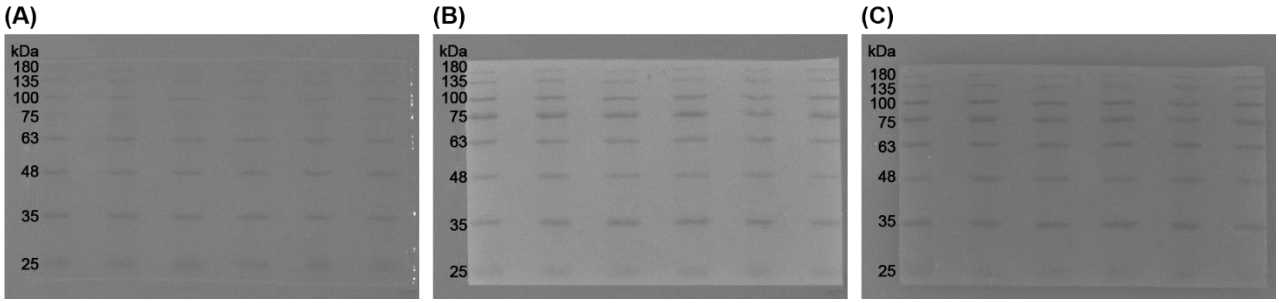


Figure 1. Pictures of (A) Gel before transfer, (B) Membrane after transfer, and (C) CBB stained membrane at different transfer times of 41, 42, and 43 minutes. All images were captured with a 0.08 s exposure time using the white LED of the KETA GLX system.

DISCUSSION

Yrdimes is a rapid and efficient semi-dry blotting device, with a minimum transfer time of 7 minutes in optimal conditions (customers can discover the best conditions for fast semi-dry transfer in Wealtec' Application Note E-03). However, this article only determines the minimal transfer time for a small-molecule-weight protein marker under normal conditions by using Bjerrum and Schafer-Nielsen transfer buffers.

The current in this report was set to 180 mA, which is roughly 2.5mA/cm^2. According to Wealtec's SemiDry Manual, with low-molecular weight protein (< 43 kDa), the current setting should be in the 2-4 mA/cm^2 range. The constant current setting of 2.5mA/cm^2 slightly improves transfer efficiency (Wealtec' Application Note E-01).

Figure 1 shows the gel before transfer, the membrane after transfer, and the CBB stained membrane at varied transfer times of 41, 42, and 43 minutes. It is evident that the signals on the membrane after transfer enhance as the transfer time increases. While the signals on the membrane after 41 minutes of transfer are low and weak, they become stronger and clearer after 42 minutes. The 25 kDa protein bands appears on the membrane after 43 minutes of transfer, however they are too faint.

In general, Yrdimes produces high-quality blotting results for small-molecule-weight protein markers (35–180 kDa) in 42 minutes, which is the shortest time under normal conditions.

CONCLUSSION

Yrdimes is designed with a bi-directional force patent, which facilitates electrode contact at all working areas to achieve steady blotting results. Because of the large working area, up to four mini gels can be transferred at once, saving users time. A simple and quick push lock makes the device simpler to assemble. The linked power supply, which has a high accuracy and stable output, ensures that the transfer works effectively.

Wealtec's computer-controlled lens gel documentation is called KETA GLX. KETA GLX can be used in molecular biology laboratories for gel applications because of its 16-bit B/W 5.0 M pixel camera, a five-position motorized filter wheel with a high transparency amber filter, dual U7 LED Epi light source, blue/white light, and 302/365 nm UV transilluminator.

REFERENCE

- Corkill, Gayle, et al. "Molecular Biomethods Handbook." (2008).
- Kurien, Biji T., and R. Hal Scofield. "Western blotting: an introduction." *Western Blotting* (2015): 17-30.

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