Efficiency Comparison of Wet and Semi-dry Blotting

INTRODUCTION

Invention of the semi-dry blotting system had largely improved the transfer speed than before and let users do the experiment with higher efficiency. Recently, innovational Pierce® Fast Semi-Dry Transfer Buffer even provides Yrdimes the ability to finish the whole transfer procedures of western blotting experiment within 7 minutes. With the high sensitive KETA ML imaging system, although transfer with Yrdimes can be finished within 7 minutes, the Yrdimes is promising to have similar performance with E-blotter system on the chemiluminescent detection.

EQUIPMENTS AND MATERIALS

- KETA ML imaging system (Wealtec)
- V-GES system (Wealtec)
- Blotting systems: Yrdimes (Wealtec), E-Blotter (Wealtec)
- Human MCF7 cell lysate and purified GST protein was obtained from Graduate Institute of Physiology in National Taiwan University College of Medicine.
- Antibody: 1st antibody: mouse-anti-GST, 2nd antibody: anti-mouse-IgG-HRP.
- Chemiluminescent: ECL Enhanced Chemiluminescence reagent (Millipore)
- Pierce® Fast Semi-Dry Transfer Buffer, 10X. (Thermo Scientific)

PROCEDURES

1. Western blot experiment was performed by the laboratory in Graduate Institute of Physiology in National Taiwan University College of Medicine.
2. Serial dilutions of human cell lysate were separated with 12% SDS-PAGE with followed samples: Purified GST protein with amount of 75, 38, 19, 9.4, 4.7, 2.35 and 1.17 pg.
3. After electrophoresis, transfer the protein onto the PVDF membrane with two systems including E-blotter and Yrdimes with Pierce® Fast Semi-Dry Transfer Buffer
4. E-blotter: Transfer with 100 V for an hour.

Yrdimes with fast transfer buffer: Transfer with 25 V for 7 minutes.

5. Hybridization with the 1st and then the 2nd antibody.
6. The result was presented with ECL Enhanced Chemiluminescence reagent.
7. Detect the result through KETA ML image system by using DynaView method with no binning setting.

RESULTS

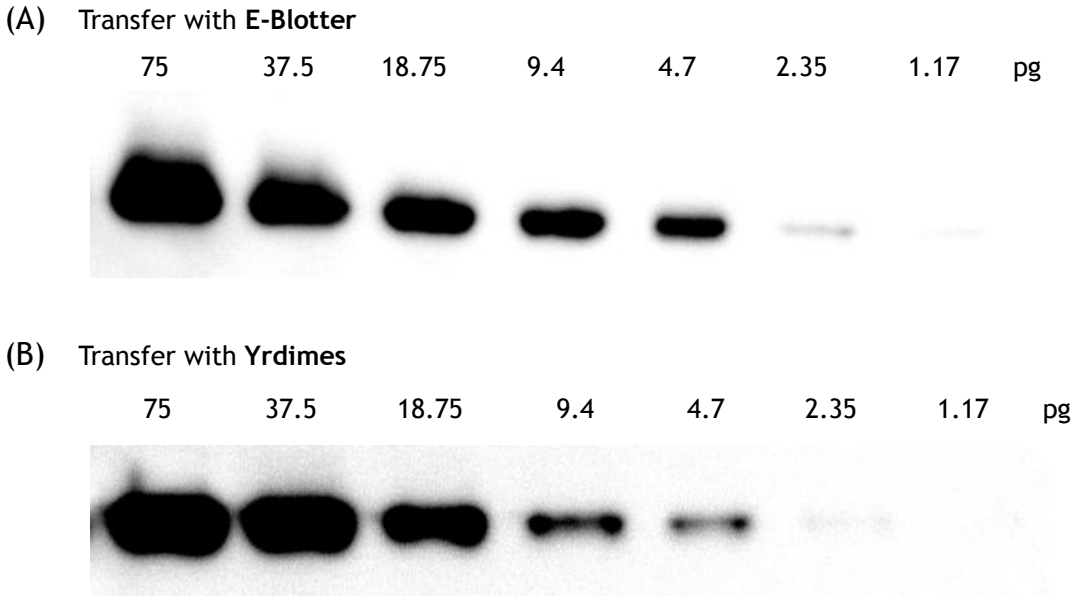

Figure 1. *Western blotting* **of** *pure GST protein transfer with (a) E-blotter and (b) Yrdimes, and detected via KETA ML imaging system. Both captured with 20 seconds DynaView exposure function for 4 pictures.*

DISSCUSION

Wet transfer with E-blotter and semi-dry transfer with Yrdimes are both well known transfer methods for western blotting. Each of them was designed for different experimental demand. While using of Yrdimes to transfer, the transferring time can be decreased to be around 40 minutes, but the common problem is that users may encounter the low transfer efficiency and resolution. Operating by the Yrdimes system with newly announced Pierce® Fast Semi-Dry Transfer Buffer can decrease the transferring time to be only 7 minutes and has no such problem on detecting of chemiluminescent samples. Under the observation with high sensitivity 2nd stage peltier cooled CCD in ML imaging system, result are as in *fig*. 1. After using of both E-blotter and Yrdimes systems to transfer the GST protein, when compared specifically on each spots, the transfer uniformity was better in E-blotter system. However, this may cause by the separation difference on the SDS-PAGE. The only thing that can be known for sure is that the chemiluminescent detection of both systems can reach at 2.35 pg when detected with the same imaging system. In order to get the result with shorter time and similar performance, it is highly recommended to use Yrdimes system with Pierce® Fast Semi-Dry Transfer Buffer to transfer their samples.

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