# Fast Semi-Dry Transfer System with Yrdimes

## INTRODUCTION

Yrdimes is a semi-dry transfer system for Western blotting mainly used for fast and efficient blotting by using less transfer buffer. It is used especially for shortening the time spent on screening bulk samples by running up to 12 pieces of 7 x 10 cm acryl-amide gels within 40 ~ 60 minutes simultaneously. Additionally, Yrdimes is equipped with platinum-coated titanium anode and stainless steel cathode plates that enable highly uniform electric field for even transfers and good for use over a long period of time.

As the semidry gel transfer system developed, there have more and more methods used for accelerating the running time during the transfer. For example, customers can modify the experimental design by replacing transfer buffers, using a different transfer apparatus, or adjusting transfer conditions to shorten the experimental time periods. Above are very useful ways for customers who need to know the result and make a decision in a short time, such as those who work for clinical inspections. As mentioned before, it takes 40 ~ 60 minutes for one transfer while using Yrdimes. But now, a high efficient protein transfer can be achieved within 7 minutes by Yrdimes blotter by replacing the conventional transfer buffer with a commercialized transfer buffer.

Transfer buffers provide an electrical continuity between the electrodes and a chemical environment to maintain the solubility of the proteins without blocking the absorption of the proteins onto the membrane during the transfer. Also, the ingredients of the transfer buffer affect the transfer time itself. Operating Yrdimes module with traditionally used buffers, it takes 40 minutes to transfer the sample. Now, in this article, with the newly announced Pierce® Fast Semi-Dry Transfer Buffer protocols, the blotting experiment have proven to work well and finish within only 7 ~ 10 minutes by Yrdimes.

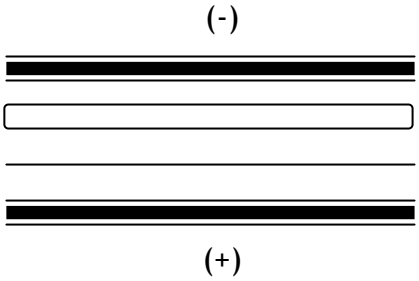
## MATERIAL

- Membranes:
  0.45 μm pore size of PVDF membrane (PolyScreen® Transfer Membrane, PerkinElmer™)
- Yrdimes (Wealtec)
- V-GES electrophoresis system (Wealtec)
- ProMarker Plus (Wealtec) and 293T cell lysate.
- Coomassie Brilliant Blue stain: 0.5 g CBB in 1000 mL MetOH: $H_2O$: AOAC = 45:45:10.
- De-staining solution: MetOH: $H_2O$: AOAC = 45:45:10.
- PVDF membrane destain solution: MetOH:AOAC = 90:10.
- Pierce® Fast Semi-Dry Transfer Buffer, 10X. (Thermo Scientific)
- Elite 200 Power Supply (Wealtec)
- Dolphin-Doc and Dolphin-Chemi mini image system (Wealtec)
- Anti-mouse ß-actin IgG antibody (Santa Cruz Biotechnology)
- Goat Anti-mouse IgG-HRP antibody (Santa Cruz Biotechnology)
- Chemiluminescence Reagent Plus (Western Lightning™)

## METHODS

- Prepare the 12% SDS-PAGE with Wealtec V-GES casting modules.
- Run the SDS-PAGE with ProMarker Plus or 293T cell lysate as samples with Elite 200. Setting of 80V and 20 minutes for the stacking gel, and 120V and 80 minutes for the resolving gel.
- In the meantime, dilute the Pierce® Fast Semi-Dry Transfer Buffer, 10 X 1:10 with deionized water and stir before use.
- Equilibrate four blotting papers (~1.25 mm) and two ultra-thick filter papers (~2.5 mm) in the diluted Pierce® Fast Semi-Dry Transfer Buffer for 10~15 minutes with gentle rocking.
- Pre-wet the PVDF membrane with methanol for 1 minute, distilled water for 3 minutes, and equilibrate in the diluted Pierce® Fast Semi-Dry Transfer Buffer for 10~15 minutes.
- After the electrophoresis is done, equilibrate the SDS-PAGE in ultrapure water for 10~15 minutes with gentle agitation.

- Transfer the SDS-PAGE into diluted Pierce® Fast Semi-Dry Transfer Buffer and equilibrate for another 10~15 minutes with gentle agitation.
- Assemble the blotting system directly on the anode plate of Yrdimes transfer unit as followed. Make sure to eliminate the bubbles between each layer in the gel-membrane sandwich.




-Cathode
-Two blotting papers with one filter paper
-SDS-PAGE Gel
-PVDF Membrane
-Two blotting papers with one filter paper
-Anode

- Close the upper lid of the Yrdimes by slightly pressing.
- Apply with 25 V constant voltages to transfer for 7~10 minutes.
- After that, stain the result as followed:

(a) Coomassie Brilliant Blue stain:

(i) Stain the SDS-PAGE and PVDF membrane with Coomassie Brilliant Blue staining solution for 30 minutes.

(ii) De-stain with different destain solutions, respectively

(iii) Document with Dolphin-Doc image system.

(b) Chemiluminescence reaction:

(i) Wash the PVDF membrane with TBST buffer for 10 ~30 minutes.

(ii) Transfer PVDF into TBST buffer with anti-mouse ß-actin IgG antibody (1:1000) for 1 hour.

(iii) Wash the PVDF membrane with TBST buffer for 10 minutes and repeat for three times.

(iv) Transfer PVDF into TBST buffer with Goat Anti-mouse IgG-HRP antibody (1:3000) for 1 hour.

(v) Wash the PVDF membrane with TBST buffer for 10 minutes and repeat for three times.

(vi) Stain with ECL Chemiluminescence reagent.

(vii) Take picture with Dolphin-Chemi mini system. Exposure with 1 minutes and integration for 10 times.

## RESULT

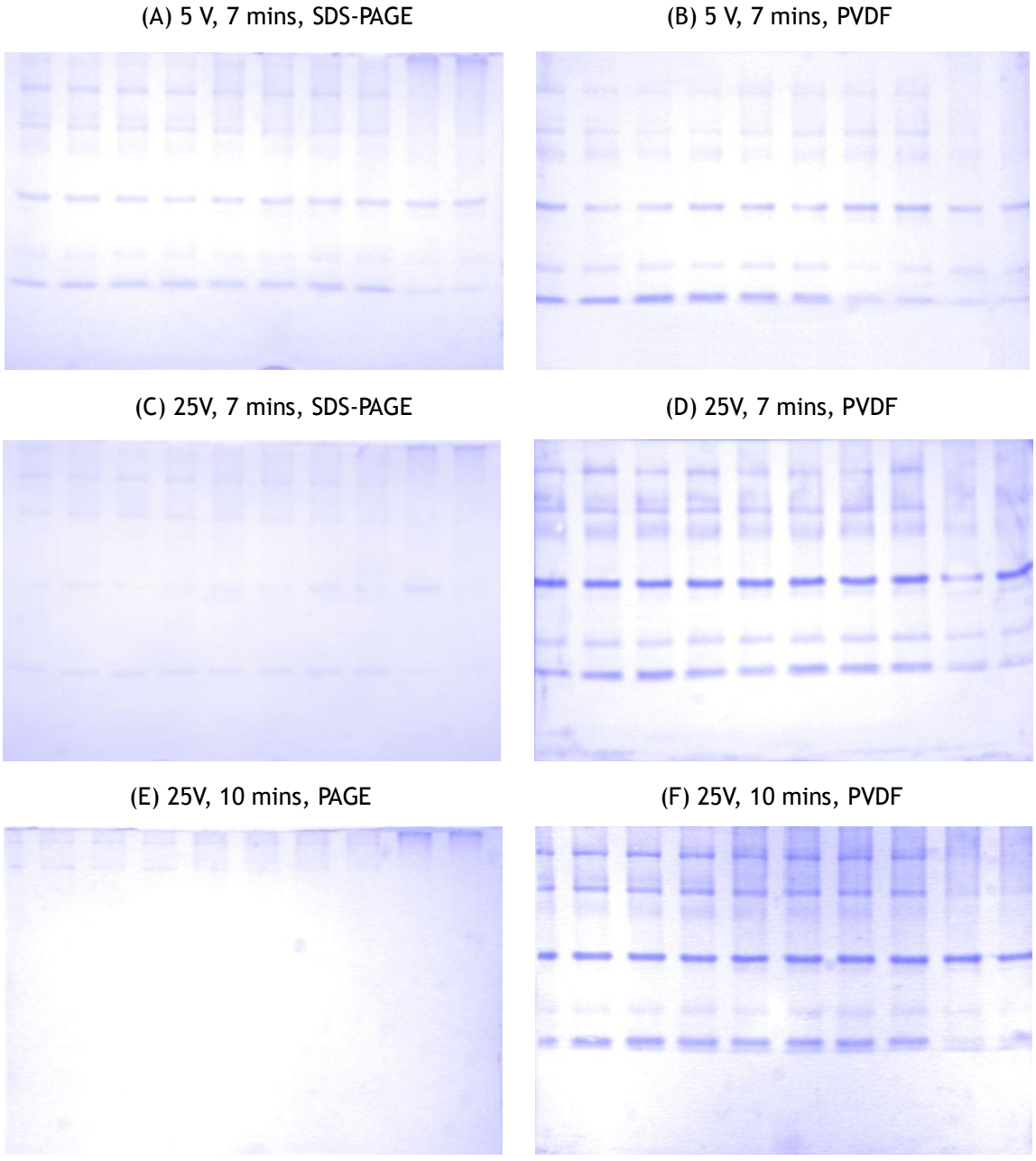



**Figure 1**. *Transfer efficiency of ProMarker Plus with different conditions. Apply with 5V (A) (B) and 25 V (C) (D) constant voltages to transfer with Yrdimes for 7 minutes, and 25 V for ten minutes (E)(F). On the left are SDS-PAGEs and PVDF membranes are on the right after transferred.*

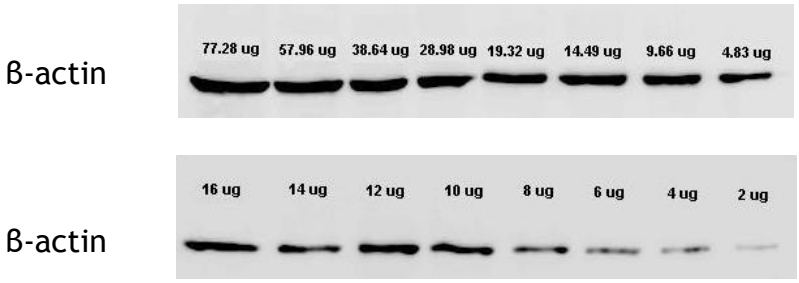



**Figure 2**. *Detection of ß-actin within series diluted 293T cell lysate after transfer from 12% SDS-PAGE to PVDF membrane with 25 V for 7 minutes. The number represent in the picture were the amount of total protein in 293T cell lysate that were loaded.*

## DISCUSSION

In the results, when applying Pierce® Fast Semi-Dry Transfer Buffer with Yrdimes, it has proved to have good transferring efficiency by applying with 25 V constant voltage and 7 minutes of transferring times. (*fig 1*) After transfer, the protein on the membrane can also be detected with the immune procedures and stain with ECL chemiluminescence reagent as in figure 2.

Comparing results that apply the different transferring constant voltages in figure 1, along with the increasing of constant voltages, it has the best transfer efficiency onto PVDF membranes while applying the maximum tolerance voltage of Yrdimes, 25 V constant voltages, which is recommended on the buffer manual. Using different transferring time lengths, it only needs seven minutes to get a very good transferring efficiency. After ten minutes transfer, up to 90% of the proteins were transferred onto PVDF membrane. Although transferring efficiency will increase by extending the operating time, it is not recommended to transfer more than 10 minutes. There is not a significant increase on the transferring efficiency after transferring more than ten minutes. Moreover, it will cause more heat production and increase the possibility of damage toward gel and samples because of applying with ultra-high constant voltage of 25 V to the Yrdimes system. As in the figure 2, the series diluted samples were decreasing in the intensity with the decreasing of the sample amount. It also proved that Yrdimes could transfer very evenly while using with Pierce® Fast Semi-Dry Transfer Buffer which would be very helpful when screening the bulk amount of samples.

In this article, it had been proven that Yrdimes was suitable to be operated with Pierce® Fast Semi-Dry Transfer Buffer to transfer the protein from the SDS-PAGE onto PVDF membrane. The result not only provides the customer a faster way to transfer protein samples, but also shows the operating flexibility of Wealtec Yrdimes system. Customers can operate the Yrdimes system in a very easy way.

## REFERENCE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