Simultaneous Semi-dry Transfer of Four Mini-size Gels

Introduction

Delivering successful large throughput western blot results is now attainable with semi-dry transfer systems. A successful western blot result is determined by the transfer efficiency and consistency. However, there are two factors affect the quality of transfer of proteins from gels to membranes using a semi-dry system: the quality of the electrode plates that keep the resistance at minimal and the stability of the power supply that is capable of operating at constant low voltage and current. To simultaneously transfer four mini-size gels, the traditional wet transfer offers high efficiency and quality but requires large volume of buffers and longer manual operation. The Yrdimes Semi-dry Transfer Systems not only offer the equivalent transfer efficiency and quality but also the flexibility with different buffers at low volume and simpler and easier operation.

Step by Step Methods and Materials

Disclaimer: the following protocol uses Yrdimes Semi-dry Transfer Unit (1073003) and ELITE 600U Power Supply (1001031). The blotting results using the power supplies from other manufacturers may be varied.

Electrophoresis Condition:

Four pieces of mini-size gels were casted with the V-GES Vertical Gel Electrophoresis Unit (V-GES, Wealtec). Each of the gels was comprised of 10% of separating gel and 4% of stacking gel. Two gel cassettes were assembled with the Electrode Module of the V-GES for electrophoresis. Two and half µl of prestained protein ladder (PageRuler Prestained Protein Ladder, Thermo) was mixed with 7.5 µl of 1X sample buffer. Each was loaded in the selected lanes for each gel. Ten µl of 1X sample buffer was loaded in the empty lanes. The gels were run for 40 minutes at 60 V and then for 70 minutes at 120 V

1. Prepare the Yrdimes Semi-dry Transfer Unit

Step	Description
1	Open the Yrdimes Semi-dry Transfer Unit by pressing the handle sides of the cathode lid with two hands simultaneously.
2	Ensure the surface of both electrode plates are clean.
3	Use the anode electrode plate for the gel-membrane assembly.

2. Prepare the Gels

Step	Description
1	After electrophoresis, remove the gels from the gel cassettes.
2	Cut away the wells or the stacking gel section.
3	Equilibrate the gels in transfer buffer for at least 10 minutes.

3. Prepare the Membranes

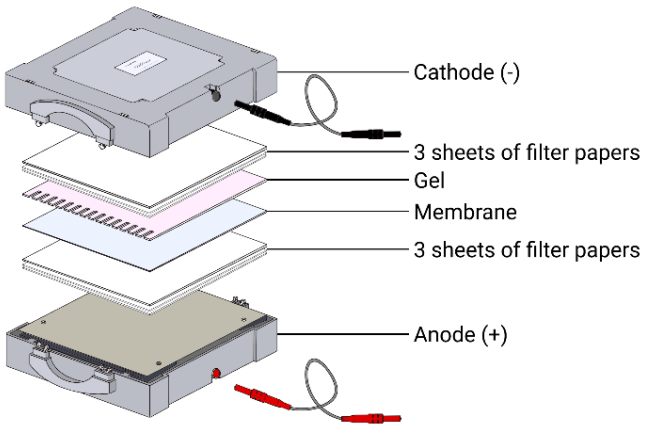
Step	Description
1	Cut the membranes to the same size as the gels.
2	If use PVDF membranes, place the membranes in methanol for 10 seconds to activate and place them in deionized water for at least 10 minutes.
3	Equilibrate the membranes in transfer buffer for at least 10 minutes.

4. Prepare the Filter Papers

Step	Description
1	Prepare six sheets of filter papers for each gel.
2	If necessary, cut all the filter papers to the same size as the membranes.
3	Equilibrate the filter papers in transfer buffer for at least 10 minutes.

5. Gel-membrane Sandwich Assembly

Step	Description
1	Assemble the gel-membrane sandwich stack one after another.
2	Use a roller or glass tube to remove air bubbles trapped in each layer.
3	Place three sheets of the prewet filter papers on the anode electrode plate.
4	Place the membrane on the filter paper stack. Remove bubbles if necessary.
5	Place the gel on the membrane. Remove bubbles if necessary.
6	Place another three sheets of the prewet filter papers on the gel.
7	Use a roller or glass tube to remove air bubbles if necessary.
8	Finish placing four gel-membrane sandwich(es) side-by-side on the anode electrode plate
9	Pour additional transfer buffer on top of each sandwich.
10	Wipe away excessive buffer around each sandwich.



6. Start Electrotransferring

Step	Description
1	Close the Yrdimes Semi-dry Transfer Unit by aligning the latch release locks on the two handle sides with the cathode lid.
2	Press down the cathode lid with two hands simultaneously.
3	Lightly lift the cathode lid and ensure it is locked in all sides.
4	Plug the cables in the semi-dry unit and ELITE 600U Power Supply.
5	Refer to Table 1 to set the transfer condition. **Note**: Do not exceed running at constant 25 volts or constant 3 mA/cm^2 for midi-size gels for more than 60 minutes. Do not exceed running for maximum four mini-size gels (6*8 cm) simultaneously.

Table 1Transfer condition according to the molecular weight of the protein of interest

Molecular Weight	Constant current	Transfer time
< 43 kDa	2 – 4 mA/cm^2	40-60 minutes
≥ 43 kDa	10 – 15 mA/cm^2	30-45 minutes

Results and Discussions

The Yirdimes Semi-dry Transfer Unit and ELITE 600U Power Supply support electrotransferring four mini-size gels simultaneously and are able to achieve outstanding results. The Yrdimes Semi-dry Transfer Unit accommodates transferring gels placed side-by-side up to 180*200 mm. The proteins from the prestained protein ladder were successfully transferred onto all four membranes (Figure1). However, the highest molecular weight protein ~180 kDa still remained in the gels (Figure 2). Optimization is still required for electrotransferring high molecular weight proteins. The ELITE Power 600U is capable of delivering constant maximum 750 mA. As a result, the voltage of the run was only shifted from 7 volts to 13 volts and the gels were able to maintain their structure after the run.

Figure 1 Two and half µl prestained protein ladder in each lane was simultaneously transferred onto four PVDF membranes from the gels.

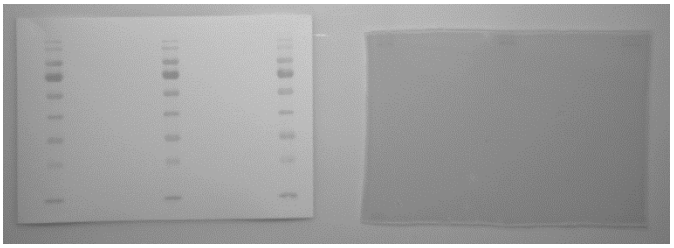


Figure 2 Only ~180 kDa protein of the prestained protein ladder in each lane was stilled remained in the gels.

Conclusion

The Yrdimes Semi-dry Transfer System achieves outstanding large throughput western blot results as it is capable of simultaneously transferring proteins from four mini-size gels to membranes. The large 180*200 mm transfer area of the electrode plates allows transferring up to two midi-size gels and up to four mini-size gels. Thanks to the accurate and stable ELITE Power Supply, operating semi-dry transfer process at low voltage and maintaining constant current allows the efficient semi-dry transfer results with a broad range of protein sizes on all four membranes.

Ordering Information

Catalog no.	Yrdimes Semi-dry Transfer Systems
1073003	Yrdimes Semi-dry Transfer Unit
1073011	Yrdimes Semi-dry Transfer System includes ELITE HC 2.0 Power Supply
1073012	Yrdimes Semi-dry Transfer System includes ELITE HC 2.5 Power Supply
1073013	Yrdimes Semi-dry Transfer System includes ELITE HC 3.0 Power Supply

Catalog no.	ELITE Power Supplies
1001021	ELITE HC 2.0 Power Supply, 5-200V, 0.01-2.0A, 100-240V, 50/60 Hz
1001023	ELITE HC 2.5 Power Supply, 2-250V 10mA-2500mA, 100-240V, 50/60 Hz
1001024	ELITE HC 3.0 Power Supply, 2-300V 0.01A-3.0A, 100-240V, 50/60 Hz
1001031	ELITE 600U Power Supply, 5-600V, 1-750mA, 120-240V, 50/60 Hz

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