Odyssey®

Infrared Imaging System

Technical Note

Protein Electrotransfer Methods and the Odyssey Infrared Imaging System

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Important: *The following methods describe suggested transfer conditions for use with the Odyssey Infrared Imager, but are intended only as a supplement to the manufacturer's instructions. Before proceeding, familiarize yourself with the appropriate user manuals and troubleshooting guidelines. Due to the large number of factors that affect protein transfer efficiency and performance, the scope of this document is limited to the materials listed in the "Required Materials" section below. Alternate materials may be substituted, if desired; however, user optimization will be necessary.*

I. INTRODUCTION

Electrophoretic elution, also referred to as electrotransfer or electroblotting, is the fastest and most reliable blotting method for transferring proteins from a polyacrylamide gel to a membrane support. The two most commonly-used techniques for electrotransfer are wet tank transfer and semi-dry transfer. This document describes the specific application of these two transfer methods for detection on the Odyssey Infrared Imager. An alternative high-speed electrotransfer method, utilizing the iBlot™ Dry Blotting System (Invitrogen Corporation, Carlsbad, CA), is also described.

Reminder: *Wear gloves at all times when handling membranes, gels, and other blotting materials. Gloves will not only prevent contamination, but also protect from exposure to potentially hazardous chemicals commonly used in blotting procedures. Avoid touching membranes directly and always use clean forceps when possible.*

II. REQUIRED MATERIALS

- Processed polyacrylamide protein gel, from one of the following sources:
  - Smart™ Gel polyacrylamide gel solutions (LI-COR Biosciences, Lincoln, NE)
    - Smart Gel 7.5% solution (LI-COR Cat. #s 928-40040 and 928-40041)
    - Smart Gel 10% solution (LI-COR Cat. #s 928-40042 and 928-40043)
    - Smart Gel 12.5% solution (LI-COR Cat. #s 928-40044 and 928-40045)
  - NuPage® Bis-Tris pre-cast gels (Invitrogen Corporation, Carlsbad, CA)
  - Novex® Tris-Glycine pre-cast gels (Invitrogen Corporation, Carlsbad, CA)
  - Ready Gel® Tris-HCl gels (Bio-Rad Laboratories, Hercules, CA)
- Odyssey Nitrocellulose Membrane (LI-COR, Cat. #s 926-31090 and 926-31092); or, Immobilon-FL PVDF Membrane (Millipore, Cat. # IPFL00010)
- 10X Tris-Glycine Transfer Buffer (LI-COR, Cat. # 928-40010)
- 10X PBS Buffer (LI-COR, Cat. #s 928-40018 and 928-40020)
- Reagent grade Methanol
- Power supply (not required for iBlot transfers)

Tank (wet) Transfer:

- Mini Trans-Blot® Electrophoretic Transfer Cell (Bio-Rad, Cat. # 170-3930)
- Blotting filter paper, thick (Bio-Rad, Cat. # 170-3932)

Semi-Dry Transfer:

- Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Cat. # 170-3940)
- Blotting filter paper, extra-thick (Bio-Rad, Cat. # 170-3967)

iBlot™ Transfer:

- iBlot Dry Blotting System (Invitrogen, Cat. # IB1001)
- For single mini-gel transfers: iBlot Gel Transfer Stack, Nitrocellulose, Mini (Invitrogen, Cat. # IB3010-02); or, iBlot Gel Transfer Stack, PVDF, Mini (Invitrogen, Cat. # IB4010-02)
- For single midi- or dual mini-gel transfers: iBlot Gel Transfer Stack, Nitrocellulose, Regular (Invitrogen, Cat. # IB3010-01); or, iBlot Gel Transfer Stack, PVDF, Regular (Invitrogen, Cat. # IB4010-01)

III. COMPARISON OF ELECTROTRANSFER TECHNIQUES

Table 1. Advantages and disadvantages of electrotransfer techniques.

	Wet tank	Semi-dry	iBlot
Advantages	Greatest flexibility for optimization	Short transfer time (15-30 minutes)	Very short transfer time (7-10 minutes)
	More complete elution of proteins	Small buffer volume requirement	Little or no buffer requirements
	Most favorable for a broader range of protein molecular weights, resulting in more consistent antibody recognition	Large surface area available for transferring large gels or several small gels	Self-contained system with very few additional components required (e.g. power supply)
	Many options available for alternate transfer equipment	Low equipment maintenance	Low equipment maintenance
Disadvantages	Longer transfer times required (1-16 hours)	Extended transfer times not possible (due to buffer depletion)	Relatively low flexibility for optimization
	Large buffer requirement (500 mL or more)	Low buffering capacity	Higher operating expense (disposable electrode stack must be purchased from Invitrogen)
	Cooling required for most systems	Variable transfer efficiencies for low and high molecular weight proteins	Transfer efficiency can vary between different proteins
	External power supply required	External power supply required	

Quantitation Comparison Examples

The following data are representative of PAGE gels transferred to a membrane support using either wet tank, semi-dry, or iBlot methods. The wet tank transfer technique generally provides more complete elution of proteins over a broad range of molecular weights. Semi-dry and iBlot transfer techniques typically give similar performance results, though semi-dry transfer allows for more optimization flexibility. See Table 1 for advantages and disadvantages of each technique.

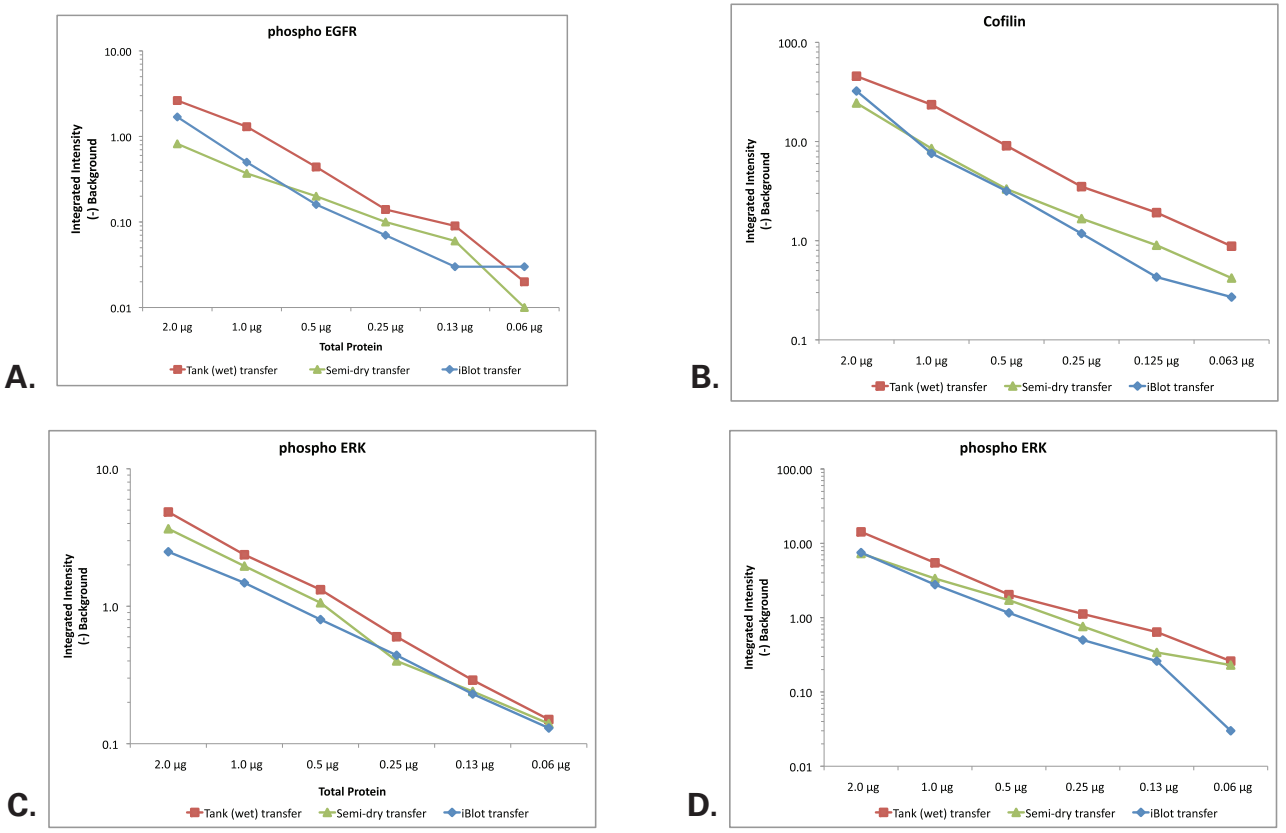

Figure 1. Western blot quantitation data comparing each of the three electrotransfer methods under varying conditions: (A) Phospho-EGFR data from NuPage 4-12% Bis-Tris gels transferred to Odyssey Nitrocellulose membranes. (B) Cofilin protein quantitation data from NuPage 4-12% Bis-Tris gels transferred to Immobilon-FL PVDF membranes. (C) Phospho ERK data from Novex 4-12% Tris-Glycine gels transferred to Immobilon-FL PVDF membranes. (D) Phospho ERK data from gels prepared with 10% Smart Gel and transferred to Odyssey Nitrocellulose.

IV. WET TANK TRANSFER using the Mini Trans-Blot System (Bio-Rad)

Description

Tank transfer is the traditional technique for electroelution of proteins to a support membrane. In this method, the gel/membrane stack is fully or partially immersed in a buffer reservoir and current is applied across the stack. Figure 2 shows a typical tank transfer setup.

Procedure

Important: *Refer to "Optimization Considerations" following this section before proceeding.*

1. Preparation for transfer:
   a. Fill the Bio-Ice cooling unit with tap water. Place unit into a -20° C freezer and allow complete freezing. After use, refill the unit and return to the freezer for future use.
   b. If necessary, cut the membrane and filter paper to the dimensions of the gel. For best results, the membrane and filter paper should be slightly larger than the gel.
   c. Prepare 1 liter of 1X transfer buffer. For improved heat dissipation during transfer, chill to 4° C prior to transfer.

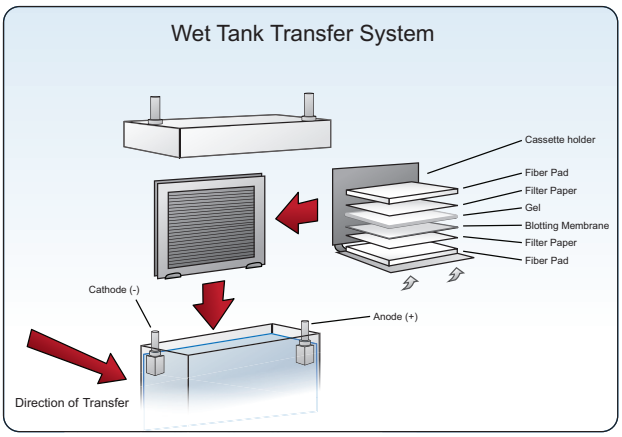

Figure 2. Diagram of Tank transfer setup.

10X Tris-Glycine Transfer Buffer	100 mL
Deionized water	700 mL
Methanol*	200 mL

* Methanol should be added last to prevent precipitation

2. After gel electrophoresis is complete, trim the wells from the gel and place the gel into a suitable tray containing 1X transfer buffer. Allow the gel to equilibrate for 15-20 minutes.

3. Soak the membrane, two sheets of pre-cut blotting filter paper, and two fiber pads in 1X transfer buffer.
   NOTE: *Wet PVDF membranes in methanol first and rinse briefly in deionized water before soaking in transfer buffer.*

4. Prepare the gel sandwich:
   a. Place the cassette holder, gray side down, on a clean, flat surface.
   b. Place one of the pre-wetted fiber pads on the gray side of the cassette.
   c. Place one sheet of wetted filter paper onto the fiber pad.
   d. Carefully place the equilibrated gel onto the filter paper.*
   e. Place the pre-wetted membrane on the gel. Ensure that the membrane completely covers the gel, leaving no gel material protruding from the edges.*
   f. Place the second sheet of wetted filter paper on the membrane.*
   g. Place the second wetted fiber pad on top of the stack to complete the sandwich.
   h. Close and latch the cassette, being careful not to disturb the gel sandwich.
   i. Place the cassette into the electrode module, with the gray side of the cassette facing the black side of the module. This ensures that the gel is closest to the cathode (-) and the membrane is closest to the anode (+).

   * Roll out any bubbles which may have formed using a blotting roller, glass tube, or similar tool.

5. Place the electrode module, the frozen Bio-Ice cooling unit, and a magnetic stir bar into the buffer tank.

6. Fill the buffer tank with cold 1X transfer buffer and place the tank onto a magnetic stir plate. Set to stir rapidly to ensure even ion distribution throughout the transfer.

7. Attach the lid and plug the cables into the power supply. Set the power supply to run at a constant voltage of 100 V for 1 hour.

8. At the end of the transfer run, disassemble the gel sandwich. Discard the polyacrylamide gel and used transfer buffer according to your facility's waste disposal guidelines.

9. For greatest sensitivity, remove the membrane to a clean, dry container and allow drying for 1 hour.

10. Wet the membrane in 1X PBS buffer and proceed with blocking and the remaining antibody incubation steps (Wet PVDF membranes in Methanol first, followed by brief rinsing in deionized water, before rinsing in 1X PBS buffer). Refer to LI-COR Bioscience's Protocol "Western Blotting Methods" for details on performing Western Blot detection on the Odyssey (available at biosupport.licor.com).

11. Clean the buffer tank, electrode module and other transfer equipment according to the manufacturer's instructions.

Optimization Considerations

Wet tank transfer allows for the greatest amount of optimization flexibility of the three electrotransfer methods described here. For this reason, the above protocol should be used only as a starting point for optimizing conditions for your particular sample. Detailed optimization guidelines are beyond the scope of this Technical Note; however, consider the following factors when beginning your optimization:

- *Buffer composition:* Typically, Towbin transfer buffer (25 mM Tris, 192 mM Glycine, 20% Methanol (v/v), pH 8.3) works well for most applications. This formulation provides a high buffering capacity, even for longer transfers, and promotes protein binding to the membrane. Alternative buffer components may be necessary for certain downstream applications such as protein sequencing, or for proteins that require a different pH for efficient transfer (due to unusually low or unusually high isoelectric points, for example).
- *Methanol:* Methanol in the transfer buffer helps to prevent gel swelling, which can produce uneven or fuzzy bands; also, methanol promotes protein binding to the membrane, particularly for nitrocellulose. Methanol can have negative effects as well, including gel pore size reduction, change in protein charge, and protein precipitation. Try reducing the methanol concentration to 10% or excluding methanol altogether to improve transfer efficiency.
- *Gel equilibration:* Generally, polyacrylamide protein gels should be soaked in transfer buffer prior to transfer. Lower percentage gels (i.e. < 12%) tend to shrink in methanol, so equilibration allows the gel dimensions to stabilize prior to transfer. Also, equilibration helps to reduce the amount of SDS and other buffer salts in the gel, which can interfere with protein adsorption to the mem brane. However, in some cases where proteins are difficult to elute from the gel, the presence of SDS in the gel, and even the addition of SDS to a final concentration of 0.05-0.1% in the transfer buffer, can improve transfer efficiency. If you do not equilibrate the gel, or if you decide to equili brate the gel in the presence of SDS, consider transferring to PVDF membrane. Adverse effects on protein adsorption caused by SDS will be reduced when using PVDF.
- *Power settings and transfer time:* The high-intensity power settings given in the above wet transfer procedure allow for a short transfer time. There are two main drawbacks to this high-intensity transfer: First, cooling is required to prevent the gel and the transfer buffer from overheating; high temperature not only poses a safety hazard, but also may damage the gel/membrane and result in poor transfer efficiency. Be certain to use chilled transfer buffer and the Bio-ice unit for high-intensity transfers. Second, high electric field strength may cause small proteins to be transferred too quickly or, conversely, incomplete transfer of large proteins. Performing the transfer overnight at low voltage (30 V), may result in a more quantitative transfer over a broader range of protein molecular weights.

Sample Wet Tank Transfer Data

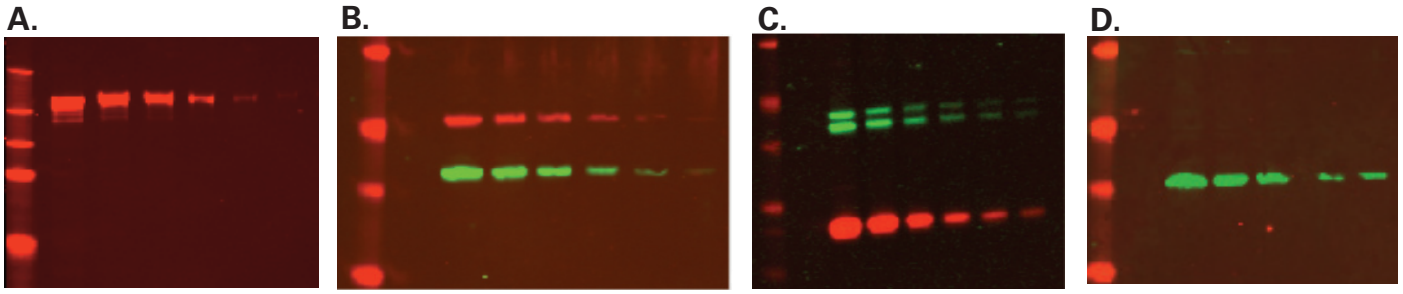

Figure 3. In each of the experiments shown above, serial dilutions of A-431 cell lysate (protein concentration range 2.0 – 0.06 µg) were loaded onto polyacrylamide protein gels, electrophoresed and transferred to membrane using the mini Trans-Blot (Bio-Rad) wet tank transfer system. Detection was performed using IRDye labeled secondary antibodies and the Odyssey Infrared Imager. (A) Phospho EGFR. (B) Tubulin (red, 700 channel) and ERK2 (green, 800 channel). (C) Cofilin (red, 700 channel) and phospho ERK (green, 800 channel). (D) GAPDH.

V. SEMI-DRY TRANSFER using the Trans-Blot® SD System (Bio-Rad)

Description

Semi-dry transfer utilizes two plate electrodes, which come in direct contact with the gel/membrane transfer stack, for electrical transfer of proteins to a membrane support. In this system, two pieces of buffer-soaked blotting filter paper replace the buffer tank and serve as the ion reservoir for current flow. Figure 4 shows a typical semi-dry transfer setup.

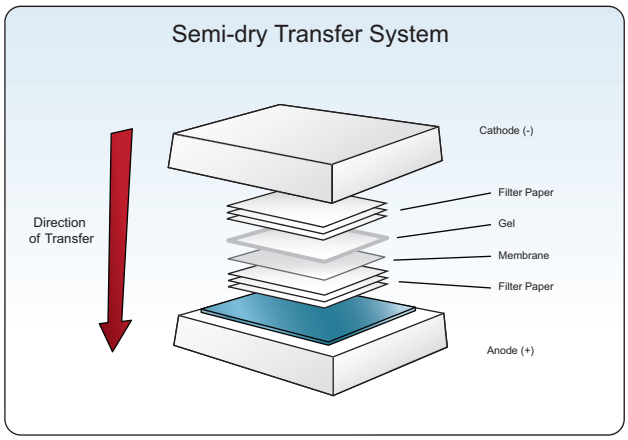

Figure 4. Diagram of semi-dry transfer setup.

Procedure

Important: *Refer to "Optimization Considerations" following this section before proceeding.*

1. Preparation for transfer:
   a. Prepare 250 mL of 1X transfer buffer. For improved heat dissipation during transfer, chill to 4° C prior to transfer.

10X Tris-Glycine Transfer Buffer	25 mL
Deionized water	175 mL
Methanol*	50 mL

* Methanol should be added last to prevent precipitation

   b. Cut the membrane and two sheets of extra-thick blotting filter paper to the dimensions of the gel(s).

      The membrane and filter paper should be the same size as, or slightly larger than, the size of the gel(s) to be transferred for best results. Filter paper that is cut too large will result in inefficient current flow through the gel.

2. After gel electrophoresis is complete, trim the wells from the gel and place the gel into a suitable tray containing 1X transfer buffer. Allow the gel to equilibrate for 15-20 minutes.

3. Soak the membrane and two sheets of extra-thick blotting filter paper in 1X transfer buffer.
   NOTE: *Wet PVDF membranes in methanol first and rinse briefly in deionized water before soaking in transfer buffer.*

4. Remove the safety cover and cathode assembly and prepare the gel sandwich:
   a. Place a sheet of pre-soaked extra thick filter paper onto the platinum anode surface.*
   b. Place the pre-soaked membrane on top of the filter paper.*
   c. Carefully place the equilibrated gel on top of the membrane. Ensure that the gel is centered on the membrane, so that no part of the gel extends past the edges of the membrane.*
   d. Place the other sheet of pre-soaked filter paper onto the gel.*
   e. Carefully place the cathode onto the transfer stack. Press down on the cathode assembly to engage the latches, making certain not to disturb the stack.
   f. Replace the safety cover and plug the unit into a power supply.

* Roll out any bubbles which may have formed, using a blotting roller, glass tube, or similar tool.

5. Set the power supply to run at a constant voltage of 20 V for 20 minutes (single mini gel), or 20 V for 30 minutes (two or more mini gels).

6. At the end of the transfer run, carefully remove the safety cover and cathode assembly. Disassemble the gel sandwich and discard the polyacrylamide gel and filter paper sheets according to your facility's waste disposal guidelines.

7. For greatest sensitivity, remove the membrane to a clean, dry container and allow drying for 1 hour.

8. Wet the membrane in 1X PBS buffer and proceed with blocking and the remaining antibody incubation steps (Wet PVDF membranes in Methanol first, followed by brief rinsing in deionized water, before rinsing in 1X PBS buffer). Refer to LI-COR Bioscience's Protocol "Western Blotting Methods" for details on performing Western Blot detection on the Odyssey (available at biosupport.licor.com).

9. Clean the cathode assembly and the base/anode assembly according to the manufacturer's recommendations.

Optimization Considerations

Direct contact of the plate electrodes with the gel/membrane sandwich in the semi-dry transfer system results in relatively faster transfers, but allows for less flexibility for optimizing transfer conditions. Consider the following factors for optimizing semi-dry transfer:

- *Transfer Buffer:* The amount of transfer buffer required is greatly reduced in the semi-dry transfer system. Also, since the electrodes are in direct contact with the transfer stack and the distance between the electrodes is relatively small, high electric field strengths can be achieved. However, the buffering capacity of the system is low and, since there is no means for external cooling, transfer time is limited. Although Towbin transfer buffer (25 mM Tris, 192 mM Glycine, 20% Methanol (v/v), pH 8.3) is suitable in most cases, alternate transfer buffers should be considered for optimizing transfer efficiency. For example:
  - Bjerrum and Schafer-Nielsen transfer buffer (48 mM Tris, 39 mM glycine, 20% Methanol (v/v), pH 9.2)
  - Discontinuous buffer system: Semi-dry transfer confers the unique ability to use different buffers for each set of filter papers in the transfer stack. One good example of this system involves using a Tris-CAPS-methanol buffer on the anode side and a Tris-CAPS-SDS buffer on the cathode side (60 mM Tris, 40 mM CAPS, pH 9.6, plus either 15% methanol or 0.1% SDS).
- *Gel equilibration:* Some electrophoresis components, particularly SDS, increase the conductivity of the transfer buffer and thereby increase the amount of heat generated during transfer. Hence, gels should generally be equilibrated in transfer buffer prior to transfer to remove these residual components.
- *Electrode contact:* Efficient transfer significantly depends on complete contact of the two electrodes with the gel/membrane transfer stack. When preparing the stack, ensure that the membrane and filter paper sheets are trimmed to the dimensions of the gel (as described in the above procedure), and that bubbles are completely removed while assembling each piece of the stack. Do not use more than the recommended amount of filter paper in the stack. A stack that is too thick will result in excessive cathode pressure on the stack. The total thickness of filter paper used in the stack should be approximately 5.0 – 7.5 mm.
- P*ower settings and transfer time:* The low buffering capacity and high amount of heat generated in semi-dry transfers necessitates a short (15 – 30 min.) transfer time. Because of the high electric field strength that can be generated, however, very efficient protein elution can be achieved. A number of factors dictate the optimal power settings and transfer time, including buffer composition & pH, gel composition, gel percentage, gel thickness, number of gels, and protein molecular

weight. Power conditions may require optimization when any of these factors changes significantly. If overheating is a problem, consider running the semi-dry transfer under constant current for a longer time (30 – 60 min.), rather than constant voltage for a short time. Under constant voltage, the current will drop off and cause the power and heat generation to increase. Maintaining constant current will decrease the amount of heat generated, although proteins will transfer slower. Refer to the manufacturer's instructions for recommended power settings.

Sample Semi-Dry Transfer Data

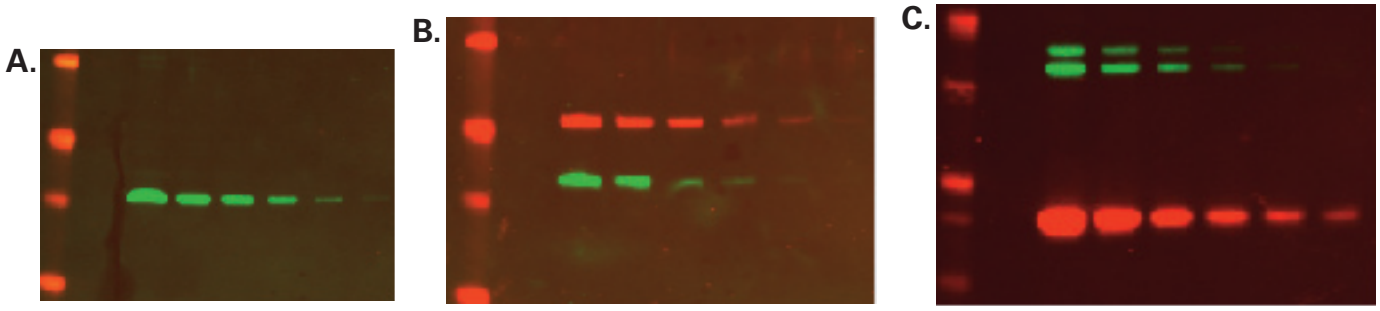

Figure 5. In each of the experiments shown above, serial dilutions of A-431 cell lysate (protein concentration range 2.0 – 0.06 µg) were loaded onto polyacrylamide protein gels, electrophoresed and transferred to membrane using the Trans-Blot SD (Bio-Rad) semi-dry transfer system. Detection was performed using IRDye labeled secondary antibodies and the Odyssey Infrared Imager. (A) GAPDH. (B) β Tubulin (red, 700 channel) and ERK2 (green, 800 channel). (C) Cofilin (red, 700 channel) and phospho ERK (green, 800 channel).

VI. iBLOT™ DRY BLOTTING TRANSFER SYSTEM (Invitrogen)

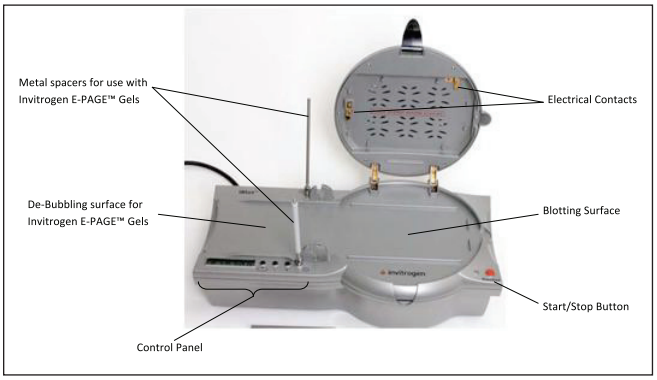

Figure 6. iBlot Gel Transfer Device

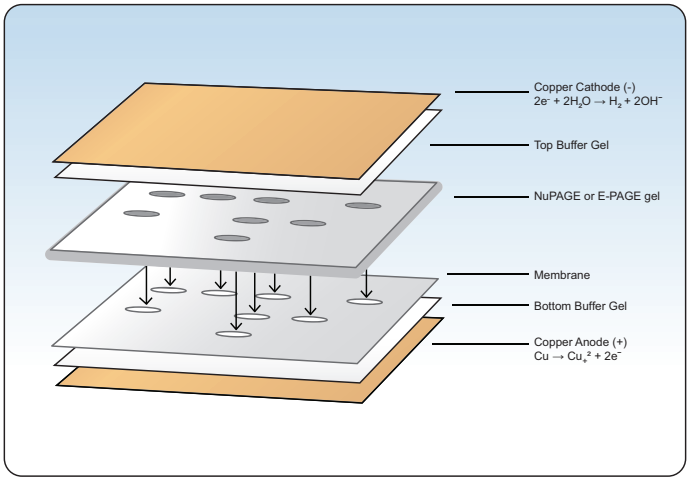

Figure 7. Gel/membrane sandwich with anode and cathode stacks

Description

The iBlot Dry Blotting System combines a patented gel matrix technology with a self-contained blotting unit to provide very fast, dry blotting of proteins to a support membrane. The concept of the iBlot system is similar to that of semi-dry electrotransfer, except that anode and cathode buffers contained in a solid gel matrix are used instead of buffer-soaked filter sheets. The iBlot device is shown in Figure 6. Figure 7 shows the transfer stack in a standard iBlot setup.

Procedure

Important: *Refer to "Optimization Considerations" following this section before proceeding.*

1. Place the device on a stable flat surface, plug in the power cord, and turn on the power switch.

2. Soak the desired membrane in 1X PBS buffer.
   NOTE: *Wet PVDF membranes in methanol and rinse briefly in deionized water before soaking in 1X PBS buffer.*

3. Open the lid of the device.

4. Remove the sealing from the anode stack, and place the anode stack onto the blotting surface, with the tray tab facing toward the right.

5. Remove the membrane from the top of the anode stack using a pair of forceps. Replace with a piece of Odyssey Nitrocellulose or Immobilon-FL PVDF membrane cut to the same dimensions.

6. Carefully place the pre-run gel on the transfer membrane, ensuring that the gel does not protrude over the edges of the membrane. One mini gel can be placed onto Anode stack, mini; or, two mini gels can be placed onto Anode stack, regular.

7. Wet a sheet of iBlot filter paper in deionized water, and place on top of the pre-run gel(s). Roll out any bubbles which may have formed using the provided Blotting Roller.

8. Remove the sealing from the cathode stack and discard the red plastic tray.

9. Place the cathode stack, with the buffer gel facing down, onto the filter paper. Roll out bubbles using the Blotting Roller.

10. Remove the disposable sponge from its wrapper, and place it on the lid of the iBlot device. The metal contact should be in the upper right corner of the lid.

11. Close the lid of the device and secure the latch.

12. Select the appropriate program and run time:
    a. For NuPage Bis-Tris gels, select program "P2" and set the run time to 10:00 minutes.
    b. For all other gels, select program "P3" and set the run time to 7:00 minutes.

13. Press the Start/Stop button. The red light changes to green, indicating the start of the run.

14. At the end of the transfer run, the device will automatically shut off and begin beeping. Press the Start/Stop button to complete the run.

15. Open the lid of the iBlot device, and disassemble the transfer stack. Discard the anode stack, gel, filter paper, and cathode stack according to your facility's waste disposal guidelines.

16. For greatest sensitivity, remove the membrane to a clean, dry container and allow drying for 1 hour.

17. Wet the membrane in 1X PBS buffer and proceed with blocking and the remaining antibody incubation steps (Wet PVDF membranes in Methanol first, followed by brief rinsing in deionized water, before rinsing in 1X PBS buffer). Refer to LI-COR Bioscience's Protocol "Western Blotting Methods" for details on performing Western Blot detection on the Odyssey (available at biosupport.licor.com).

18. Turn off the iBlot power switch. Clean the blotting surface and lid with a clean, damp cloth or paper tissue, and store the device according the manufacturer's recommendations.

Optimization Considerations

Invitrogen's iBlot™ transfer technology uses a similar concept as semi-dry transfer, but replaces the user-formulated buffer system with a patented, high-performance buffer gel matrix to achieve very fast transfers. Limited flexibility for changing transfer conditions restricts the amount of user optimization necessary, but the following factors should be considered:

- *Power settings and transfer time:* Five transfer programs are available, which correspond to different voltage settings (10 – 25 V). Program selection, together with transfer time adjustment, has a considerable impact on transfer efficiency. The instrument settings given in the above procedure will typically provide good results in most cases; however, adjustments may be required, depending on the type and percentage of gel being used. When optimizing conditions, begin by adjusting transfer time up or down in increments of 30 seconds. As a general rule, decrease the voltage by one step for every 2-3 minutes of time added to the run. Refer to the manufacturer's instructions for detailed optimization guidelines.
- *Gel equilibration:* One of the advantages of the iBlot system is that transfer buffer is not generally necessary. However, in some cases, more efficient protein elution may be achieved with gel equilibration. Prepare 100 mL of transfer buffer in a shallow tray, and allow the pre-run gel to soak for 5-15 minutes.

10X Tris-Glycine Transfer Buffer	10	mL
Deionized water	70	mL
Methanol*	20	mL

* Methanol should be added last to prevent precipitation

- *Electrode contact:* Complete contact between the anode stack, membrane, gel, and cathode stack is essential to achieving optimal transfer efficiency. Ensure that all components of the transfer stack are aligned evenly, and that bubbles are completely removed during assembly. Also, be sure to remember to include the disposable sponge, and to secure the latch when closing the lid on the device. The disposable sponge and electrode stacks may be used only once.
- *Membrane choice:* Odyssey Nitrocellulose or Immobilon-FL PVDF membranes give the best results for detection on the Odyssey Infrared Imaging System. The membrane provided with the anode stack is compatible with the Odyssey System, but higher background signal may result. When replacing the provided membrane, be sure to cut the new membrane to the appropriate dimensions and wet it in transfer buffer or deionized water prior to placing it onto the anode stack (wet PVDF membrane in methanol first, followed by soaking in transfer buffer or deionized water). No preparation is required if using the Invitrogen membrane provided with the anode stack.

Sample iBlot Transfer Data

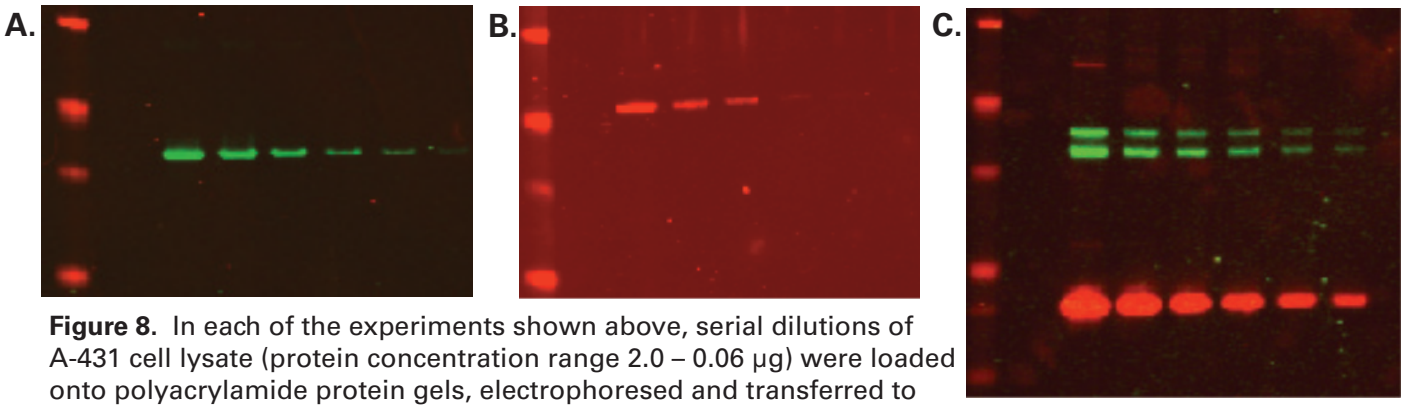

Figure 8. In each of the experiments shown above, serial dilutions of A-431 cell lysate (protein concentration range 2.0 – 0.06 µg) were loaded onto polyacrylamide protein gels, electrophoresed and transferred to membrane using the iBlot Dry Blotting System (Invitrogen). Detection was performed using IRDye labeled secondary antibodies and the Odyssey Infrared Imager. (A) ERK2 (B) β Tubulin (C) Cofilin (red, 700 channel) and phospho ERK (green, 800 channel).

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