Video Article

Simultaneous, Rapid, and Highly Efficient Protein Transfer Using the Trans-Blot Turbo Transfer System - ADVERTISEMENT

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Abstract

Western blotting is a well established analytical technique used to detect specific proteins in a sample[1–10]. Following protein separation through gel electrophoresis, the proteins are transferred to a solid membrane support. Once on the membrane, a protein of interest can be detected with antibodies specific for the target protein[5,6]. Numerous molecular biology disciplines employ this technology on a daily basis. Although it is a common laboratory practice, western blotting is a time consuming process and technological advances leading to increased time efficiency and quality are invaluable across widespread scientific disciplines.

The Trans-Blot Turbo system reduces the time to perform protein transfers in a high throughput manner, while maintaining high efficiency transfers. The base unit includes an integrated power supply compatible with universal line voltage, as well as an LCD screen and keypad allowing access to operational parameters. The unit has two independently-controlled transfer cassette bays. Each cassette can transfer up to 2 mini gels or a single midi gel simultaneously. The top lid of each transfer cassette is composed of a stainless steel cathode fixed to a spring-loaded plate to ensure that equal and consistent pressure is applied to the transfer sandwich. The bottom tray contains an anode plate made of platinum-coated titanium. From the software interface, optimized preloaded protocols can be selected. Protocols can also be customized and there is space for up to 25 user-defined transfer protocols to be saved and recalled.

While the system can be used with standard blotting reagents for traditional semi-dry transfer, Trans-Blot Turbo transfer packs are necessary for rapid transfer applications. Specialized precut filter paper and PVDF or nitrocellulose membranes are supplied pre-saturated with optimized buffer. The combination of the specialized filter paper and optimized buffer offers higher ion capacity than standard blotting reagents, enabling higher currents to be passed through the transfer stack to speed up protein migration and improve transfer efficiency. Furthermore, there is no need to equilibrate the gel in transfer buffer. When the Trans-Blot Turbo system is used in combination with the Bio-Rad Mini-PROTEAN TGX gels, efficient protein transfer can be performed in three minutes, significantly reducing the time involved in applying transfer technology.

Here, we present a 3-minute transfer protocol, as well as a protocol to simultaneously transfer 4 mini gels in as little as 7 minutes.

Video Link

The video component of this article can be found at http://www.jove.com/video/3158/

Protocol

1. Three-Minute Protein Transfer

1. Following gel electrophoresis using Mini-PROTEAN TGX gels, open the Trans-Blot Turbo cassette lid by turning the knob counterclockwise until a small click is felt.
2. To begin assembly of the blotting sandwich, open a mini turbo transfer pack. The mini transfer pack tray contains two adjacent ion reservoir stacks. The top stack is on the left and the bottom stack is on the right. Each stack can also be identified by the labeling on the tray, with the bottom stack holding the membrane.
3. Place the bottom stack in the bottom tub of the cassette, with the membrane facing up. Using the blot roller, roll the stack and membrane gently to remove any air trapped between the membrane and the cassette.
4. Position the gel on top of the membrane; equilibration of the gel in transfer buffer is not necessary. Roll the gel with the blot roller to remove bubbles.
5. Next, lay the top stack on the gel, and roll again with the blot roller. Place the cassette cover onto the sandwich and press down firmly. Using the symbols on the lid as a guide, lock the cassette by turning the knob clockwise until the cassette clicks.
6. Insert the locked cassette into the top "A" bay of the Trans-Blot Turbo transfer instrument.
7. Access the TURBO protocol by pressing the "TURBO" button on the keypad below the LCD screen. Then press the key next to "1 MINI TGX." Next, press "A:RUN." In the run screen, real-time voltage, amperage conditions, as well as a countdown timer will be displayed.

8. "A" bay and "B" bay are independently controlled; hence the user can insert the cassette into either bay as long as it is available.
9. If necessary, a second 3-minute transfer can be performed simultaneously in the independently controlled bottom "B" bay.
10. Once the run is complete, the unit will emit a beep and "RUN COMPLETE" will display on the LCD screen.
11. Remove the cassette from the unit and open. First remove the top stack, and then the transferred blot.
12. After removing all the stack components, clean the cassette by rinsing and wiping with a paper towel.
13. The blot should be detected at a convenient time.

2. Simultaneous Protein Transfer of up to Four Mini Gels in 7 Minutes

1. Up to four mini gels can be transferred using the Trans-Blot Turbo system with 2 midi transfer packs or 4 mini transfer packs. Alternatively, each midi transfer pack can be used with one midi-sized gel.
2. In the Midi transfer packs, the top and bottom ion reservoir stacks can be found in one tray, one on top of the other. Using the tabs in each upper corner, the top and bottom stacks can be easily separated.
3. To assemble a transfer sandwich, use the right tab to lift up the bottom stack, which contains the membrane. Place the stack in the bottom tray of an open cassette with the membrane facing up. Roll the stack and membrane gently with the blot roller to remove any air bubbles.
4. After placing the bottom stack in the cassette base, take the first previously run mini gel and place it on the left side of the midi-format stack. Align the top of the gel with the outer edge of the stack in such a way that the low molecular weight proteins are near the center of the stack.
5. Take a second previously run mini gel and position it on the right side of the stack in the opposite direction, so that the top of the gel again is aligned with the outer edge of the stack. Use the blot roller to remove air bubbles from the gels.
6. Next, remove the top stack from the tray and place it on top of the gels. Use the roller again. Lock the cassette cover.
7. Insert the locked cassette into the upper "A" bay of the Trans-Blot Turbo transfer instrument.
8. In the same manner, prepare the second cassette using 2 additional previously run mini gels. Insert the second cassette into the lower "B" bay.
9. Access the TURBO protocol by pressing the "TURBO" button on the keypad below the LCD screen. Then press the key next to "2 MINI or 1 MIDI gel." Next, press "A:RUN." Repeat this process for the cassette in the "B" bay.
10. After the run is complete, remove the transferred blots for further processing.
11. As before, clean up by rinsing the cassette and then wiping it down with a paper towel.

3. Representative Results

Using the Trans-Blot Turbo system, a single Mini-PROTEANTGX gel (for proteins with MW 5–150 kD) can be transferred in as little as 3 min. Up to 4 mini or 2 midi gels with mixed-molecular weight proteins (MW 5–150 kD) can be efficiently transferred in 7 min. For high-molecular weight proteins (MW 25–300+ kD), up to 4 mini or 2 midi gels can be efficiently transferred in 10 minutes (**Figure 1**).

E. coli lysate (6 µg) samples diluted two-fold were separated with Mini-PROTEAN TGX gels, transferred with the Trans-Blot Turbo system, stained with SYPRO Ruby protein gel stain and imaged on a VersaDoc MP 4000 imaging system. Standards in lane 1 are Precision Plus Protein Unstained, with a top band of 250 kD MW (**Figure 1**).

Trans-Blot Turbo transfer outperforms current blotting techniques as visualized by increased signal intensity using Trans-Blot Turbo system compared to other traditional tank, semi-dry blotting, and dry blotting techniques (**Figure 2**).

Signal intensities after transfer were calculated to be 6x stronger using the Trans-Blot Turbo system, relative to existing rapid transfer technology. Furthermore, the coefficient of variation (CV) across a single blot was 50% lower in comparison (**Figure 3**).

Quantitation performed on equivalent 2-D gels transferred with the Trans-Blot Turbo system as compared to the dry transfer system yielded twice the number of proteins transferred and detected with the Trans-Blot Turbo system. In 2-D spot quantitation 1,066 spots were detected with the Trans-Blot Turbo system, while only 555 spots could be detected using the other rapid transfer technology (**Figure 4**).

	Acceptable Combinations*		**Not Acceptable***	
Combination	**1**	**2**	**1**	**2**
Upper Bay A	1 mini gel	2 mini gels or 1 midi gel	1 mini gel	2 mini gels or 1 midi gel
Lower Bay B	1 mini gel	2 mini gels or 1 midi gel	2 mini gels or 1 midi gel	1 mini gel

Table 1. This table lists the acceptable conditions for protein transfer in the Trans-Blot Turbo system.

Protocol Name	**MW, kD**	**Time, min**	**2 Mini Gels or 1 Midi Gel**	**1 Mini Gel**
STANDARD SD	Any	30	Up to 1.0 A; 25 V constant	
1.5 MM GEL	Any	10	2.5 A Constant; up to 25 V	1.3 A constant; up to 25 V
HIGH MW	>150	10		
LOW MW	<30	5		
MIXED MW*	Any	7		
TURBO Mini TGX	Any	3	N/A	2.5 A constant; up to 25 V

* Also accessed via the TURBO Navigation button.

Table 2. This table lists the preprogrammed protocols provided in the Trans-Blot Turbo system.

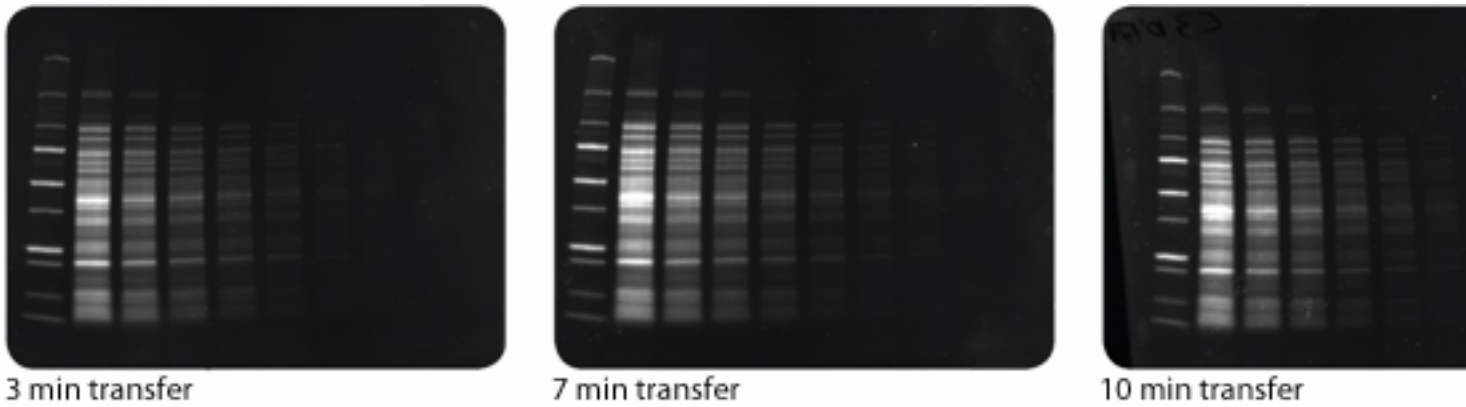

Figure 1. *E. coli* lysate (6 µg) was diluted two-fold. Samples were separated with Mini-PROTEAN TGX gels, transferred with the Trans-Blot Turbo system, stained with SYPRO Ruby and imaged on a VersaDoc 4000 MP system. Standards in lane 1 are Precision Plus Protein Unstained, with a top band of 250 kD MW.

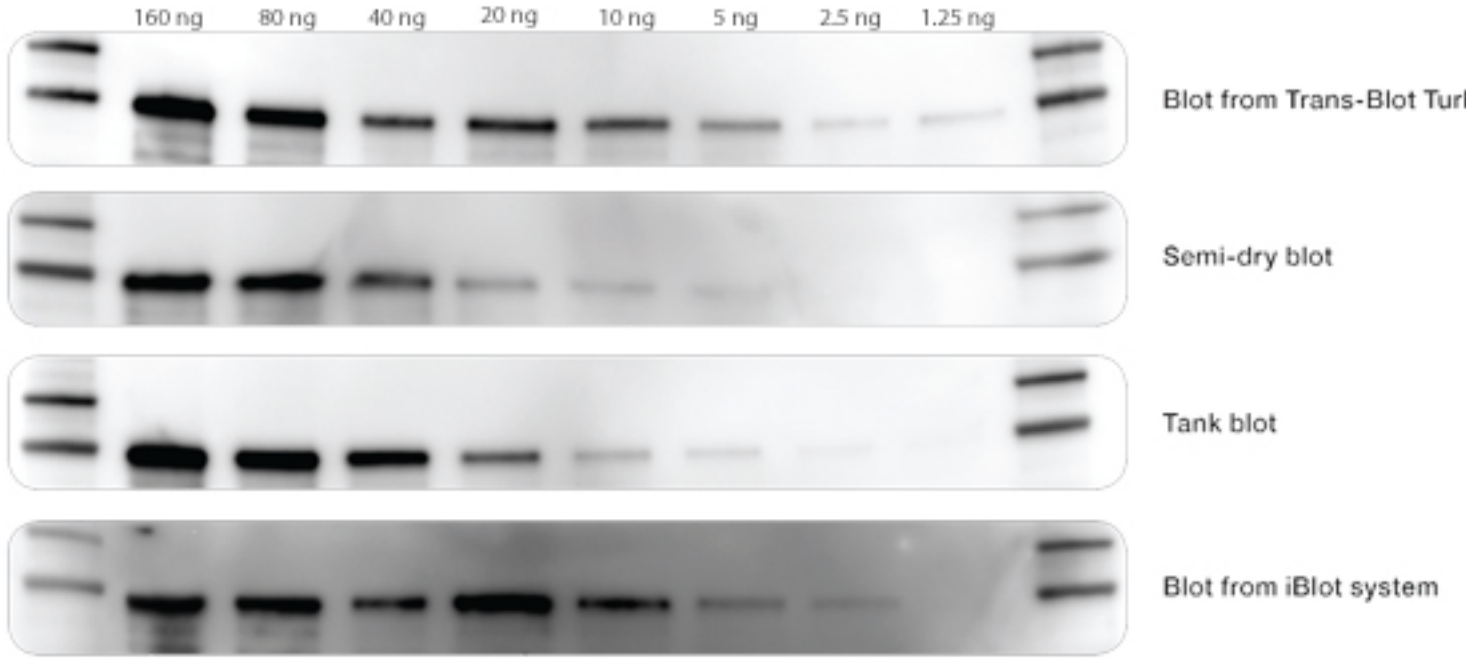

Figure 2. Comparison of Trans-Blot Turbo Transfer to traditional tank, semi-dry blotting, and dry blotting techniques.

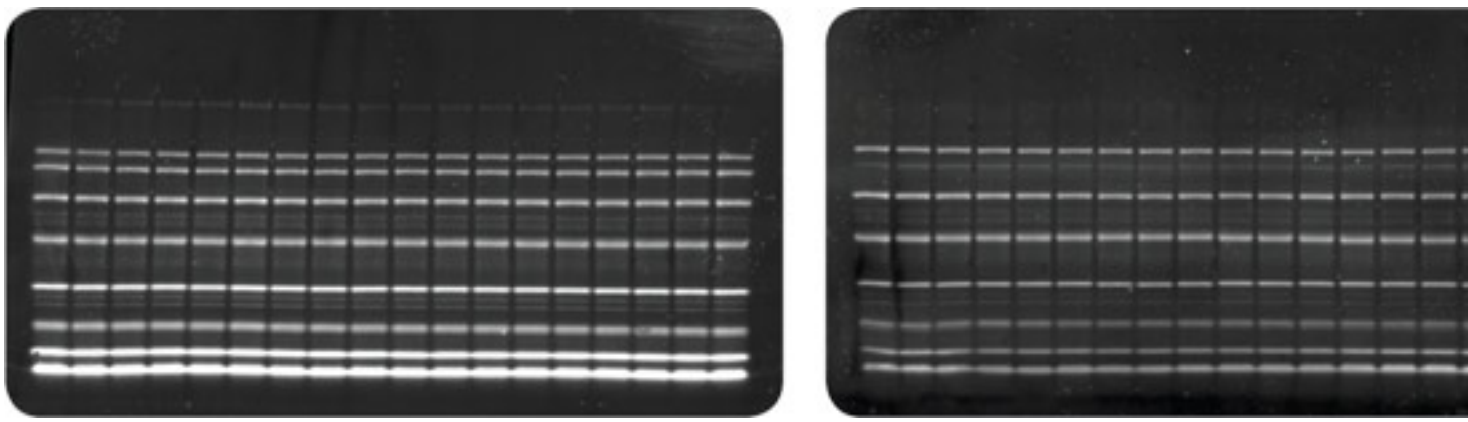

Figure 3. Signal intensities after the transfer were calculated to be 6x stronger with the Trans-Blot Turbo as compared to a rapid dry blotting system. Coefficient of variation (CV) across a single blot was 50% lower with the Trans-Blot Turbo system than with a dry blotting system.

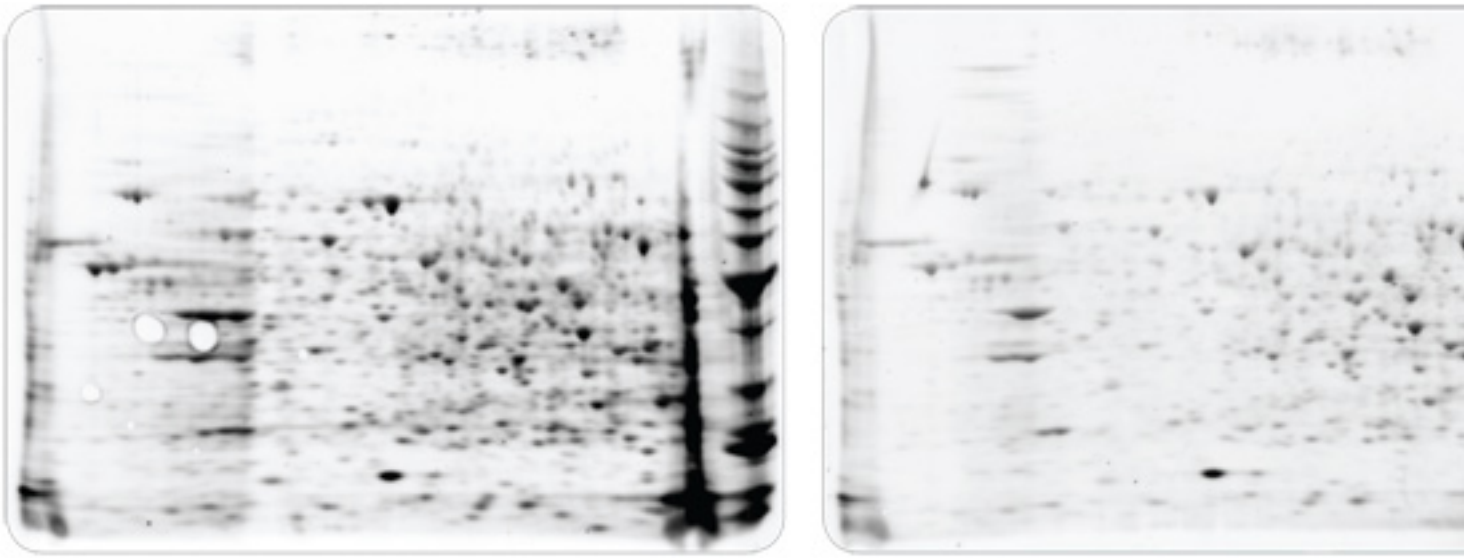

Figure 4. 2-D spot quantitation performed on equivalent 2-D gels transferred with Trans-Blot Turbo and dry blot systems yielded 1066 spots with the TBT system and 555 spots with the dry blot system.

Discussion

Specific detection and analysis of target proteins from a mixture of proteins separated using SDS-PAGE is primarily achieved through transfer of the protein from the gel matrix to a membrane where it can be detected using specific antibodies. Since the advent of this technology, numerous transfer techniques have been implemented[11,12], including the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets[1]. The latter was introduced in 1979 and exploits the electrophoretic mobility of proteins to transfer them; it has become routine in today's laboratory due to its speed and transfer efficiency.

Electrophoretic transfer can be accomplished using three different methods, which utilize the same principles, including wet, semi-dry, and dry transfer systems. The wet transfer system was the original technique introduced by Towboin[1], and involves the gel/membrane stack being completely immersed in a buffer reservoir where current is then applied. Though historically the most efficient protein transfer method, it is also the most costly in terms of time and reagents. The semi-dry transfer system, described by Kyhse-Anderson[13], replaces the buffer reservoir with layers of filter paper soaked in buffer sandwiched between graphite plate electrodes, providing a constant current density and decreasing the time and reagents necessary for efficient transfer. One caveat to this technique is that it is generally not as effective for high molecular weight proteins. Dry transfers have recently been used to give fast transfers, but at the cost of a lower transfer efficiency. The balance of transfer speed and efficiency has been an ongoing consideration as new techniques are introduced in an effort to optimize protein transfer.

Described here is a modification of the semi-dry system, in which optimization has resulted in decreased time and increased transfer efficiency, particularly for high molecular weight proteins. The Trans-Blot Turbo Transfer System utilizes new buffer and filter paper formulations to allow much higher amperage to be driven through the blot, effectively reducing the blot run time to as little as 3 minutes.

While the preprogrammed protocols efficiently transfer most proteins, some proteins may require further optimization. For example, poor electrophoretic transfer with excessive protein remaining in the gel may be due to a transfer time that is too short. In particular, high molecular weight proteins (>150 kDa) may require increased transfer time. In such cases, the transfer time should be increased in 1 min increments; optimization of the voltage and current should also be performed. A critical step in using the Trans-Blot Turbo Transfer system is to correctly assemble the blotting sandwich. Failure to perform this step properly can result in poor electrophoretic transfer in conjunction with the loss of protein in the gel. It is also critical to use the blot roller to ensure that air bubbles or excess buffer are removed, as poor contact between the membrane and the gel due to air bubbles can result in swirls, missing bands, or diffuse transfers. For optimal results, this technology is designed to be coupled with the supplied reagents. However, the Trans-Blot Turbo system is not limited to the use of these products and can be used in conjunction with other reagents (Table 1). Although the unit is supplied with numerous preprogrammed transfer protocols (Table 2), it can be easily programmed with user-defined protocols as well.

As demonstrated in this protocol, the Trans-Blot Turbo system reduces the transfer time as compared to currently available protein transfer technologies, while surpassing the quality of the results and allowing for the simultaneous transfer of several blots for increased efficiency.

Disclosures

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