Robust Transfer of Proteins to Membranes Across a Wide Range of pI and Mass Using the Trans-Blot® Turbo™ System

ChengJun Sun, Karen Zhu, Tom Berkelman, Jeff Xu, and William Strong
Bio-Rad Laboratories, 6000 James Watson Drive, Hercules, CA 94547

Introduction

Western blotting is a powerful tool for protein detection and characterization. It involves the transfer of electrophoretically separated protein from a gel matrix onto a porous membrane with subsequent immunodetection of the protein bound to the surface of the membrane. Efficient and reliable protein transfer is a key factor in determining the success of the process.

The efficiency of protein transfer depends on many factors, including the properties of the proteins, the nature of the gels, the transfer system, the composition of the transfer buffer, and the type of membrane used. Typically, proteins are transferred from native or SDS-PAGE gels to nitrocellulose or PVDF membranes by different means, such as electrophoretic transfer (Towbin et al. 1979), diffusion (Renart et al. 1979) and vacuum blotting (Peferoen et al. 1982). The most commonly used method is electrophoretic transfer; it is performed either by immersing the transfer sandwich in a buffer tank (tank transfer) or by placing a buffer-soaked transfer sandwich between two plate electrodes (semi-dry or SD transfer). Tank transfer is considered the gold standard of the blotting techniques because reliable and quantitative transfers are achieved. Semi-dry blotting systems provide the researcher with a faster and simpler process compared to the tank blotting method. However, the high electrical field strengths and reduced transfer buffer volumes required can result in blow-through of low molecular weight proteins and/or poor transfer of high molecular weight proteins.

Bio-Rad's Trans-Blot Turbo transfer system is based on an improvement of the typical semi-dry blotting approach. The system significantly reduces the blotting time and increases the transfer efficiency over the conventional SD system, giving equivalent or better results compared to the widely used tank blotting process when used with Bio-Rad's TGX gels. In this study, we tested the performance of the Trans-Blot Turbo transfer system with different gel types; we also compared its performance with tank transfer and Invitrogen's iBlot system using a rat liver sample separated on 2-D gels to compare a wide range of proteins with different isoelectric points (pI) and masses.

Methods

Samples

Frozen rat liver (Pel-Freez Biologicals) was homogenized in a sample solution composed of 8 M urea, 4% CHAPS, 40 mM DTT, and 0.2% (w/v) Bio-Lyte® ampholyte 3–10 at a ratio of 1:8 (w/v). The homogenized sample was centrifuged at 14,000 × g for 15 min and the supernatant was reserved for the 2-D electrophoresis.

2-D Electrophoresis

The first-dimension separation was conducted using 7 cm or 11 cm pH 5–8 ReadyStrip™ IPG strips (Bio-Rad Laboratories, Inc.). The rat liver sample was diluted in a rehydration buffer consisting of 7 M urea, 2 M thiourea, 2% CHAPS, 0.2% (w/v) Bio-Lyte ampholyte pH 5–8, 50 mM DTT, and 0.001% (w/v) bromophenol blue dye to give a final concentration of 0.5 mg/ml. Diluted samples were loaded via passive rehydration in volumes of 125 µl and 185 µl for the 7 cm and 11 cm IPG strips, respectively. The first-dimension focusing for the 7 cm strips was run with a program of 250 V for 30 min followed by 4,000 V until 25,000 Vh was reached. The current limit was 50 µA. The 11 cm strips were focused with a program of 250 V for 30 min followed by 8,000 V for 35,000 Vh under a current limit of 50 µA.

Following first-dimension isoelectric focusing (IEF), the focused IPG strips were equilibrated for 15 min in equilibration buffer I (6 M urea, 375 mM Tris-HCl pH 8.8, 2% (w/v) SDS, 30% glycerol, 2% (w/v) DTT) followed by another 15 min in equilibration buffer II (6 M urea, 375 mM Tris-HCl pH 8.8, 2% (w/v) SDS, 30% glycerol, 2.5% (w/v) iodoacetamide). Second-dimension separations of 7 cm IPG strips were performed on 4–20% Mini-PROTEAN® TGX™ precast gels, and 11 cm IPG strips were performed on Criterion™ Tris-HCl or Criterion™ TGX™ gels. Equilibrated strips were applied and mounted to the second-dimension gels with agarose and run in Tris-Glycine-SDS buffer at 200 V until the dye front reached the bottom of the gel. NuPAGE Novex 4–12% Bis-Tris and Novex 4–20% Tris-Glycine Zoom gels (Life Technologies) were used for second-dimension separations of 7 cm IPG strips in an XCell SureLock mini-cell according to the manufacturer's recommended protocol. The focused 7 cm strips were first incubated for 15 min in the manufacturer's reducing buffer (1× LDS buffer with reducing agent) followed by another 15 min in the LDS buffer with iodoacetamide added. The equilibrated strips were applied to the gels following the manufacturer's recommendation and run in MOPS buffer (NuPAGE gels) or Tris-Glycine-SDS buffer (Tris-Glycine gels) at 125 V until the dye almost ran to the end of the gel.

Blotting

Following second-dimension SDS-PAGE, the gels were blotted onto either nitrocellulose or PVDF membranes according to standard protocols for each blotting system as listed in Table 1.

Blot Staining

Following protein transfer, blots were first fixed in 7% acetic acid/10% methanol for 15 min followed by four ddH_2O rinses of 5 min each. Blots were then stained with 25 ml (midi-size) or 10 ml (mini-size) SYPRO Ruby stain. After the blots were rinsed six times with ddH_2O, they were imaged using a VersaDoc™ 4000 MP imaging system using either blue or green LED excitation and a 605 nm bandpass emission filter.

Gel Staining

Following blotting, the gels were fixed in 40% methanol/10% acetic acid for 30 min and then stained with Bio-Safe™ Coomassie stain for at least 1 hr. After destaining with water, the gels were scanned on a Molecular Imager® GS-800™ calibrated densitometer using standard settings.

Results

Trans-Blot Turbo Performance with a Variety of Gel Chemistries and Comparison with iBlot

To evaluate the robustness of the Trans-Blot Turbo system for protein transfer over a wide range of molecular mass and intrinsic charge, a rat liver sample focused on pH 5–8 IPG strips was resolved on second-dimension gels with different chemistries and blotted onto nitrocellulose membranes using both the Trans-Blot Turbo and iBlot systems. The gel chemistries used for second-dimension separation included: Criterion Tris-HCl 8–16% precast gel (Figure 1), Criterion™ TGX Any kD Stain-Free™ precast gel (Figure 2), 4–20% Mini-PROTEAN® TGX™ precast gel (Figure 3), NuPAGE Novex Bis-Tris 4–12% precast gel (Figure 4), and Novex 4–20% Tris-Glycine precast gel (Figure 5). In order to compare signal levels across all the blots and gels, images were transformed to the same extent.

Table 1. Blotting conditions used for the different systems tested.

System	Trans-Blot Turbo System	iBlot System	Tank Blotting	XCell SureLock Mini-Cell
Transfer Conditions	Mini gels: 25 V/1.3 A for 7 min Midi gels: 25 V/2.5 A for 7 min	Mini and midi gels 25 V for 7 min (Protocol: P3)	Mini Trans-Blot® Cell: 100 V for 1 hr, 2 hr, >16 hr (overnight) Criterion™ Blotter: 100 V for 30 min (plate electrodes)	25 V for 1 hr
Transfer Buffer	Trans-Blot Turbo transfer packs	Proprietary transfer stacks	Towbin buffer with 20% methanol	2× NuPAGE transfer buffer

Criterion Tris-HCl 8–16% precast gel — This gradient percentage was chosen because it is the most popular midi size gel used for 2-D electrophoresis. Utilizing the traditional Tris-HCl formulation, this gel gives the best resolution between 10 to 100 kD and is suitable for most complex biological samples.

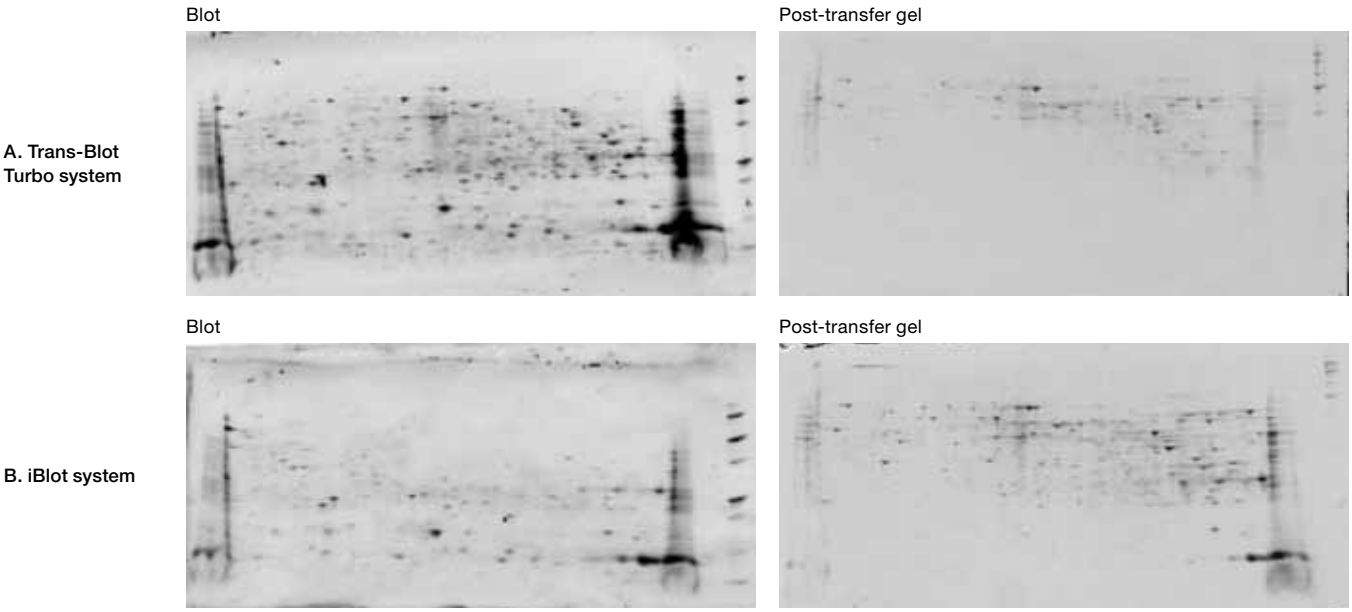


Fig. 1. Comparison of transfer efficiency of Criterion Tris-HCl 8–16% gels using the Trans-Blot Turbo and iBlot blotting systems. Gels were transferred onto nitrocellulose membranes using the Trans-Blot Turbo system (**A**) or the iBlot system (**B**). SYPRO Ruby–stained blots (left panels) and Coomassie-stained post-transfer gels (right panels) are shown.

Criterion TGX Any kD Stain-Free precast gel — This gel uses Bio-Rad's proprietary TGX formulation with the Stain-Free feature, allowing fast visualization of protein bands upon UV activation using the Bio-Rad Gel Doc™ EZ or ChemiDoc™ MP imaging systems. TGX gels perform similar to Tris-HCl gels, but they provide the added benefit of a one year shelf life. We selected this gel because the unique Any kD format shows the best resolution for proteins below 100 kD and is the recommended Criterion TGX Stain-Free gel for most 1-D and 2-D applications.

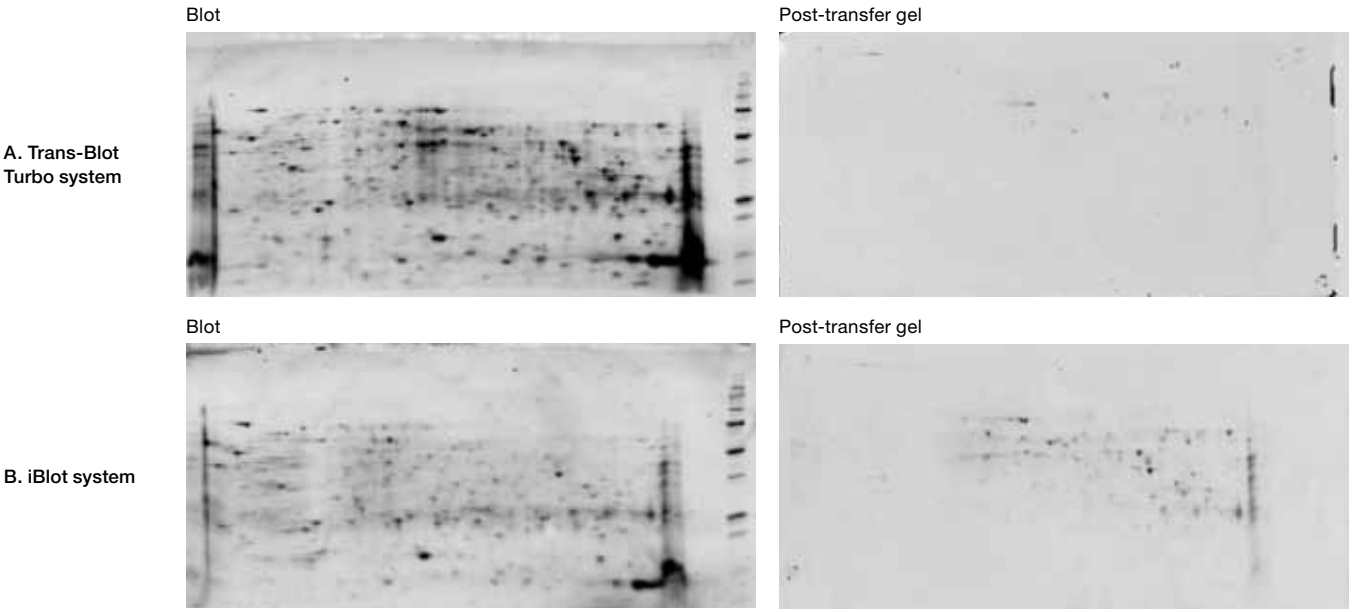


Fig. 2. Comparison of transfer efficiency of Criterion TGX Any kD Stain-Free gels using the Trans-Blot Turbo and iBlot blotting systems. Gels were transferred onto nitrocellulose membranes using the Trans-Blot Turbo system (**A**) or the iBlot system (**B**). SYPRO Ruby–stained blots (left panels) and Coomassie-stained post-transfer gels (right panels) are shown.

Mini-PROTEAN TGX precast gel, 4–20% — The Mini-PROTEAN TGX precast gel is a mini gel format of the Criterion TGX precast gel. As with any TGX gel, this precast gel is compatible with Laemmli sample buffer and Tris/glycine-based running buffer; it also has a one year shelf life compared to the 3 month shelf life associated with Tris-HCl–based precast gels. We selected this gel because the mini format is the preferred gel size by researchers and 4–20% is the most popular gradient gel percentage.

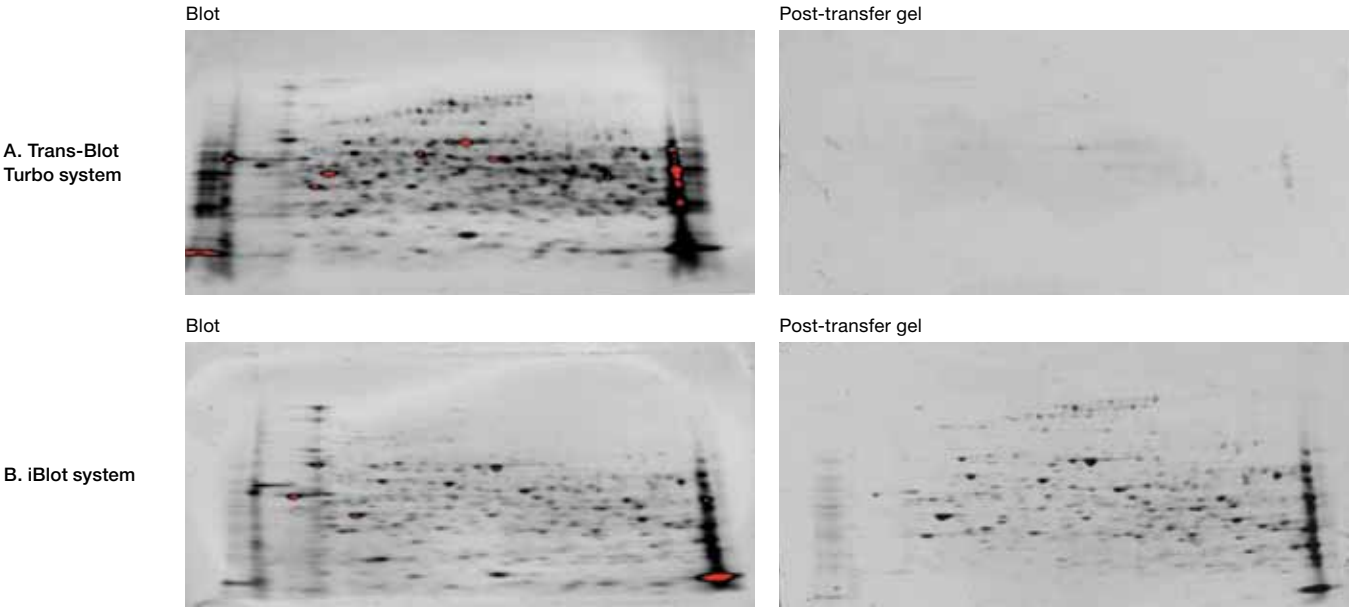


Fig. 3. Comparison of transfer efficiency of 4–20% Mini-PROTEAN TGX precast gels using the Trans-Blot Turbo and iBlot blotting systems. Gels were transferred onto nitrocellulose membranes using the Trans-Blot Turbo system (**A**) or the iBlot system (**B**). SYPRO Ruby–stained blots (left panels) and Coomassie-stained post-transfer gels (right panels) are shown.

NuPAGE Novex Bis-Tris 4–12% precast gel — Life Technologies' NuPAGE Bis-Tris precast gels use a proprietary neutral pH formulation that provides a one year shelf life. It is not compatible with traditional Tris/glycine-based running buffer; therefore, researchers must use specialized MOPS or MES running buffers for this gel. The separation pattern is also quite different compared to a Laemmli system precast gel. We selected this precast gel because NuPAGE Bis-Tris precast gels are quite popular with researchers and the gel formulation could affect gel transfer efficiency.

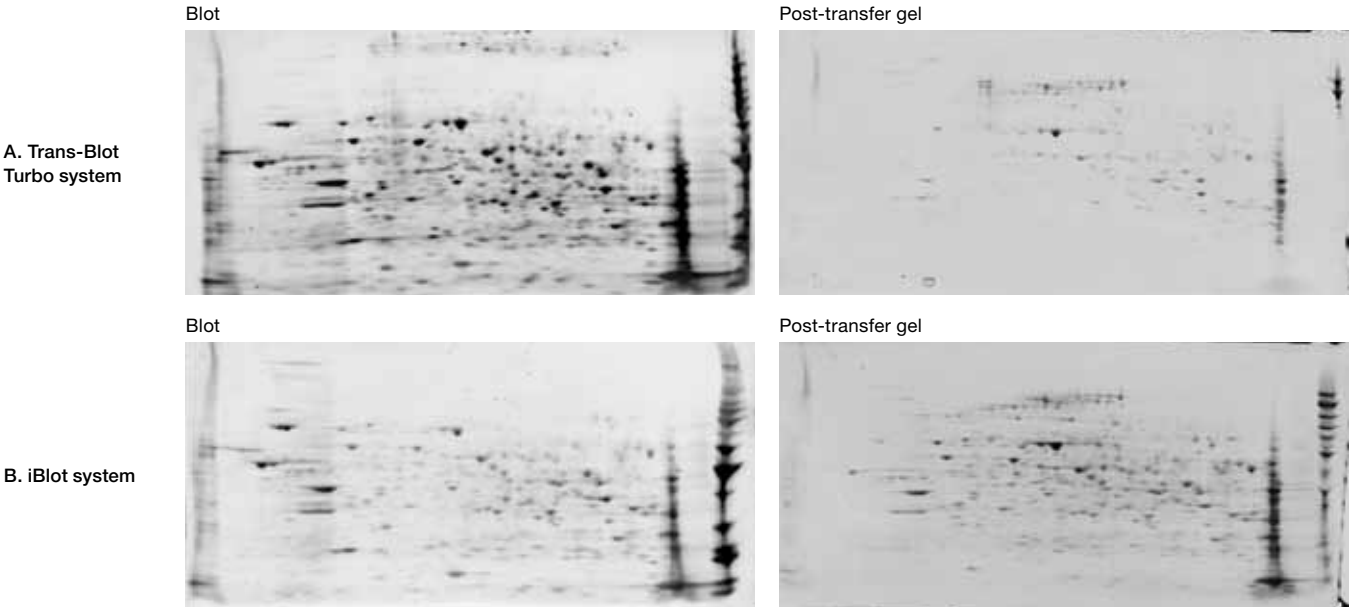


Fig. 4. Comparison of transfer efficiency of NuPAGE Novex 4–12% Bis-Tris gels using the Trans-Blot Turbo and iBlot blotting systems. Gels were transferred onto nitrocellulose membranes using the Trans-Blot Turbo system (**A**) or the iBlot system (**B**). SYPRO Ruby–stained blots (left panels) and Coomassie-stained post-transfer gels (right panels) are shown.

Novex Tris-Glycine 4–20% precast gel — The Novex Tris-Glycine precast gel is another precast gel from Life Technologies. We selected another Tris-HCl precast gel because it eliminates the potential gel variations from different vendors. It also shows that, in terms of transfer, there are minimal variations between vendors if the same gel formulation is selected.

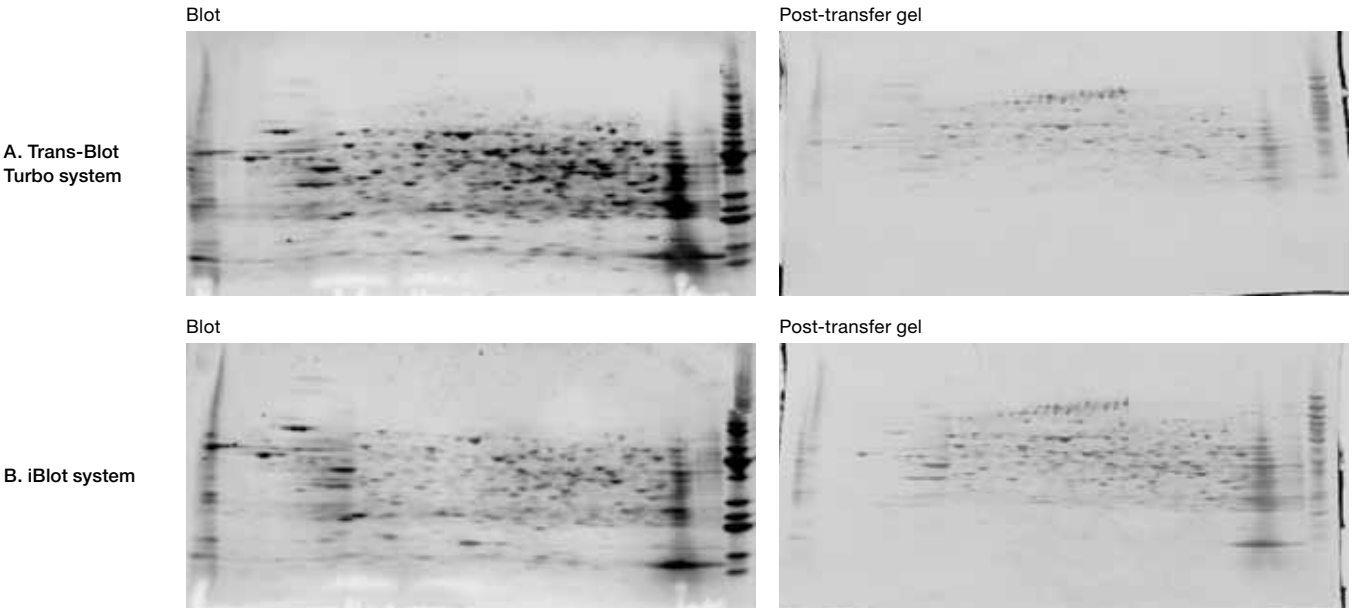


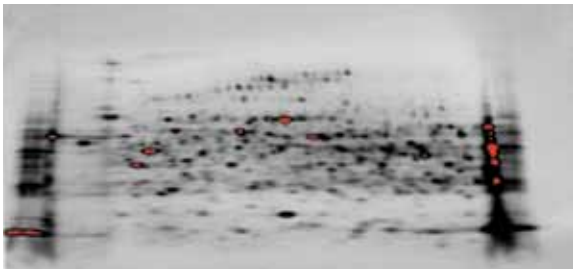
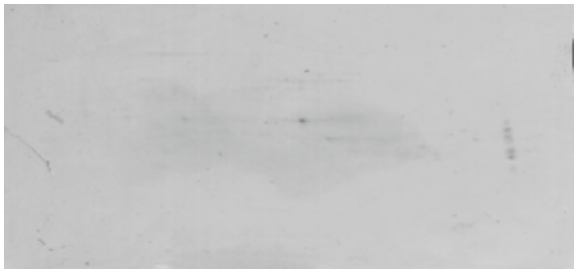
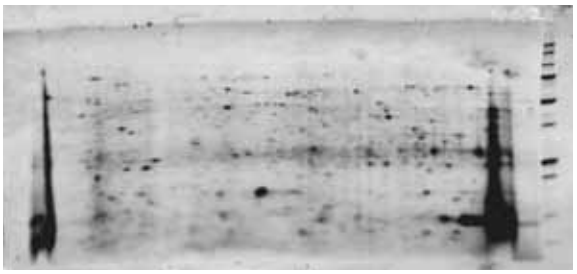
Fig. 5. Comparison of transfer efficiency of Novex Tris-Glycine 4–20% precast gel using the Trans-Blot Turbo and iBlot blotting systems. Gels were transferred onto nitrocellulose membranes using the Trans-Blot Turbo system (**A**) or the iBlot system (**B**). SYPRO Ruby–stained blots (left panels) and Coomassie-stained post-transfer gels (right panels) are shown.

As shown in Figures 1 to 5, for all the different gel types tested, the Trans-Blot Turbo system showed consistently higher blotting efficiency over a wide range of protein masses and pIs, with little or no protein remaining in the gel. Blotting efficiency of the Trans-Blot Turbo system was the highest for TGX gels. When compared with the iBlot system, the Trans-Blot Turbo system consistently demonstrated better transfer efficiencies showing higher protein intensities on the blots and much fewer and fainter protein spots on the post-transfer gels regardless of the gel type. Similar results were also observed with all the tested gel chemistries when using PVDF membranes (data not shown). Therefore, the Trans-Blot Turbo system is compatible with many different gel chemistries and consistently outperforms the iBlot system with regard to transfer efficiency.

Trans-Blot Turbo Performance Comparison against Tank Blotting Systems

A comparison of transfer efficiency was performed between the Trans-Blot Turbo system and the Bio-Rad tank systems (Mini Trans-Blot® Cell and Criterion blotter) on one hand and between the Invitrogen iBlot and the Invitrogen tank system (XCell II Tank) on the other hand. Nitrocellulose membranes were used for these comparisons. The comparison between the Trans-Blot Turbo system and the Bio-Rad tank systems was performed using 4–20% Mini-PROTEAN TGX precast gels with 7 cm focused IPG strips and Criterion TGX gels using 11 cm focused IPG strips. As shown in Figure 6 (A to F), the Trans-Blot Turbo blotting system combined with either Mini-PROTEAN or Criterion TGX gels showed blot results as good as or better than those obtained from overnight tank transfers, with similar/stronger protein intensity on the blots and almost no protein left on the gel. Visual examination of the blots revealed that a 2 hr tank transfer did not increase the transfer efficiency over a 1 hr transfer. However, overnight tank transfer at a reduced voltage resulted in more proteins transferred onto the membrane (B, C, D). When examining the post-transfer gels from these blots, 1 hr and 2 hr tank transfer conditions did not show a corresponding increase in protein remaining in the gel relative to overnight tank transfer, suggesting blow-through might occur when voltages are set high during shorter transfer periods. The Trans-Blot Turbo system, in just 7 min, achieved protein transfer efficiencies that met or exceeded those obtained using overnight tank transfer (A, D). The Invitrogen iBlot and Invitrogen tank transfer system were compared using NuPAGE Novex 4–12% Bis-Tris ZOOM gels and 7 cm focused strips. The results shown in Figure 6 (panels G to H) demonstrate that the iBlot system left considerable amounts of untransferred proteins in the gel when compared to the Invitrogen tank system. The protein spots on the iBlot-derived membrane were of significantly reduced signal intensity relative to the membrane produced using tank blotting. Our comparison of blot performance across platforms demonstrated that the Trans-Blot Turbo system produced efficient protein transfers of quality comparable to that achieved using tank transfer methods but in a fraction of the time.

4–20% Mini-PROTEAN TGX precast gel

	Blot	Post-transfer gel
A. Trans-Blot Turbo mini gel (1.3 A, 7 min)		
B. Mini Trans-Blot cell (100 V, 1 hr)		
C. Mini Trans-Blot cell (100 V, 2 hr)		
D. Mini Trans-Blot cell (30 V, 16.5 hr)		
E. Criterion blotter (100 V, 1 hr)		
F. Trans-Blot Turbo, midi gel (2.5 A, 7 min)		

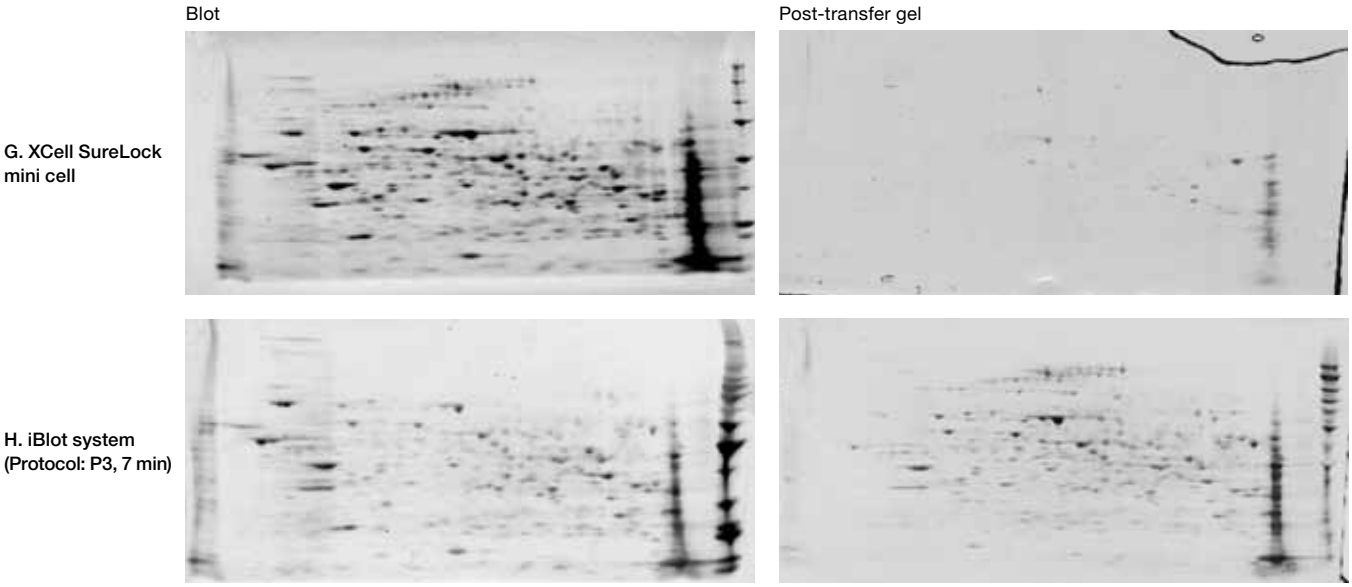


Fig. 6. Comparison of transfer efficiency of Trans-Blot Turbo and other blotting systems. 4–20% Mini-PROTEAN TGX precast were transferred using the following systems: Trans-Blot Turbo (**A**), Mini Trans-Blot for 1 hr (**B**), Mini Trans-Blot for 2 hr (**C**), and Mini Trans-Blot overnight (**D**). Criterion TGX Any kD precast were transferred using the Criterion Blotter for 1 hr (**E**) and Trans-Blot Turbo (**F**) systems. NuPAGE Novex 4–12% Bis-Tris gels were transferred using the XCell SureLock (**G**) and iBlot systems (**H**). SYPRO Ruby-stained blots (left panels) and Coomassie-stained post-transfer gels (right panels) are shown.

Conclusions

Natural protein samples contain diverse proteins with different physicochemical properties such as pI, mass, and hydrophobicity. With the added factor of different gel chemistries being used for second dimension protein separations, there are many challenges to achieve efficient blotting. Bio-Rad's Trans-Blot Turbo transfer system is based on an improvement to the Trans-Blot SD semi-dry transfer cell combined with proprietary pre-wet transfer sandwiches. The Trans-Blot Turbo transfer system was designed to accelerate the blotting process without compromising transfer efficiency. When compared with the iBlot system, the Trans-Blot Turbo system produces more efficient blotting regardless of the gel chemistry used when the same transfer time was used.

We also demonstrated that when using Mini-PROTEAN or Criterion TGX gels for second dimension separation, the Trans-Blot Turbo system performs as well as or better than traditional tank blotting methods for the transfer of proteins with different pI and molecular weight in significantly less time. The Turbo system, with its compact design, small footprint and high blotting efficiency can be readily adapted to any western blotting workflow.

References

Peferoen M et al. 1982. Vacuum-blotting: a new simple and efficient transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to nitrocellulose. FEBS. Lett. 145, 369–372.

Renart J et al. 1979. Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. Proc Natl Acad Sci USA. 76, 3116–3120.

Towbin H et al. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and applications. Proc Natl Acad Sci USA 76, 4350–4354.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 5044 5699*
Canada *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88* ***United Kingdom*** *020 8328 2000*

Bulletin 6147 Rev B US/EG *12-0440 0212 Sig 1211*