



Transferring Large and Small Proteins using the iBlot™ Dry Blotting System

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Introduction

The iBlot™ Dry Blotting system has enabled many users to achieve extremely fast protein transfers for a broad range of proteins. Pre-set conditions are recommended with the iBlot™ System using the “P3” program and 7 minute transfers. These conditions work for many protein samples, but as with any experimental procedure, working parameters may need to be optimized for your proteins of interest and their molecular weights.

The P3 program (20 volt transfer) transfers proteins of 30 kDa – 150 kDa molecular weight very well. Like in any electrophoresis separation, small proteins (under 30kDa) migrate more rapidly than large ones and in the same manner, need less time to transfer from the matrix of the gel to the membrane. While P3 program for 7 minutes works well with most proteins, less time is needed for the transfer of smaller proteins with the iBlot (P3 5-6 minutes).

Larger proteins (over 150 kDa) migrate slower than smaller proteins during sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and therefore need more time to transfer. The recommended time to transfer large proteins with the iBlot should be extended to 8-10 minutes.

In this technical note, we describe several ways to enhance transfer efficiency above and below this molecular weight range to provide optimal results. These methods include an equilibration (gel soaking) step after gel electrophoresis and before dry transfer, appropriate gel selection, and varying the protein transfer time.

Experimental Section

To make Equilibration Buffer for optimizing large protein transfer:

Use 2X NuPAGE® Transfer Buffer containing 10% methanol and 1:1000 NuPAGE® Antioxidant.

1. Create 100 ml of Equilibration Buffer for each gel.
2. Following protein electrophoresis, equilibrate (soak) the gel in transfer buffer for 10-20 minutes at room temperature on a shaker prior to transfer.
3. Remove excess liquid, place equilibrated gel on iBlot™ stacks and blot the gel with the iBlot™ device using the P3 program for 7-10 minutes.
4. Based on initial results, the transfer time can be optimized.

Gel Selection:

In order to determine the dependency of transfer efficiency on gel type used, SDS-PAGE was performed with samples using two distinct types of gradient mini gels:

- NuPAGE® Novex® 4-12% Bis-Tris Gels with MES running buffer (Invitrogen)
- NuPAGE® Novex® 3-8% Tris-Acetate Mini Gels

Large Protein Transfers (≥ 150 kDa):

To analyze large protein transfer improvement methods, a large purified protein, KLH (400-3,000 kDa), was loaded onto a 4-12% Bis-Tris gel and a 3-8% Tris-Acetate gel in the following lane order:

1. 10 ng KLH purified protein
2. 7.5 ng KLH purified protein
3. 5 ng KLH purified protein
4. 2.5 KLH purified protein
5. 1 ng KLH purified protein
6. 750 pg KLH purified protein
7. 500 pg KLH purified protein
8. 250 pg KLH purified protein
9. 100 pg KLH purified protein
10. 50 pg KLH purified protein

Small Protein Transfers (≤ 30 kDa):

To analyze small protein transfers with decreased time, small proteins (histones, 11-21 kDa), were loaded onto a 4-12% Bis-Tris gel.

1. 1 µg of Histone proteins
2. 500 ng of Histone proteins
3. 250 ng of Histone proteins
4. 100 ng of Histone proteins
5. 75 ng of Histone proteins
6. 50 ng of Histone proteins
7. 25 ng of Histone proteins
8. 10 ng of Histone proteins
9. 7.5 ng of Histone proteins

SDS-PAGE protocols and protein transfers to nitrocellulose membranes were performed according to manufacturers' instructions. Gels were transferred using the iBlot™ Dry Blotting device and an Invitrogen XCell II™ (Wet) Blot Module (Figures 1 and 2).

Immunodetection of proteins on the resulting membranes in Figure 1 was performed using a WesternBreeze® Chromogenic Immunodetection Kit (Invitrogen) for detection of rabbit primary antibodies. A rabbit anti-KLH primary antibody (Sigma) was used (1:10,000) with a chromogenic development time of 1 hour.

Immunodetection of proteins on the resulting membranes in Figure 2 was performed using a WesternBreeze® Chromogenic Immunodetection Kit (Invitrogen) for detection of rabbit primary antibodies. A rabbit anti-histone H3 primary antibody (Sigma) was used (1: 5,000) with a chromogen development time of 1 minute (chemiluminescent, CL) and 1 hour (chromogenic).

Results

Figure 1 compares the resulting protein blots after longer transfer (8 min vs. the recommended 7 min) with the iBlot™ Dry Blotting System (P3 for 8 minutes), and wet transfer (60 minutes at 30 volts) for the KLH large protein separated with NuPAGE® Novex® 4-12% Bis-Tris Gels (Figure 1 - a,b,c) and 3-8% Tris-Acetate Mini Gels (Figure 1 - d,e). The equilibrated 4-12% Bis-Tris gel (1b) shows greater sensitivity in comparison to the gel transferred in the wet system (1c) and the non-equilibrated gel (1a).

The 3-8% Tris-Acetate Mini Gel transferred with the iBlot™ device (1d) shows greater sensitivity in comparison to the 3-8% Tris-Acetate Mini Gel transferred in the wet system (1e), and compared with the Bis-Tris gel without equilibration (1a). This indicates that for large proteins, combining the iBlot™ System and Tris-Acetate gels may be more successful than the use of Bis-Tris gels or wet transfer techniques.

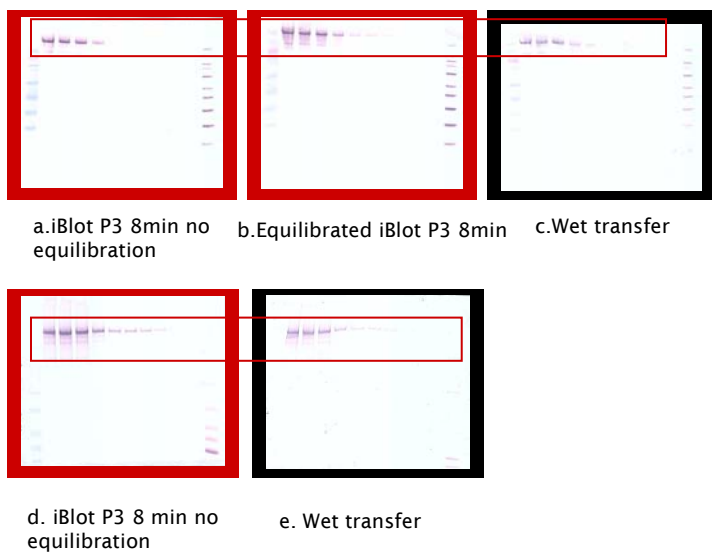


Figure 1. Membranes from NuPAGE® Novex® 4-12% Bis-Tris Gels (1a, b, c) and 3-8% Tris-Acetate Mini Gels (1d,e) with KLH purified protein (~220 kDa) transferred with the the iBlot™ Dry Blotting device and an Invitrogen XCell II™ Blot Module

Figure 2 compares the resulting protein blots after short transfer (6 min vs. the recommended 7 min) with the iBlot™ Dry Blotting System (P3 for 6 minutes) (a,b), and wet transfer (c,d) for the small Histone protein separated with NuPAGE® Novex® 4-12% Bis-Tris Gels.

The dry transfer membrane data is visually comparable to the wet transfer data and sensitivity is sustained between dry and wet blotting.

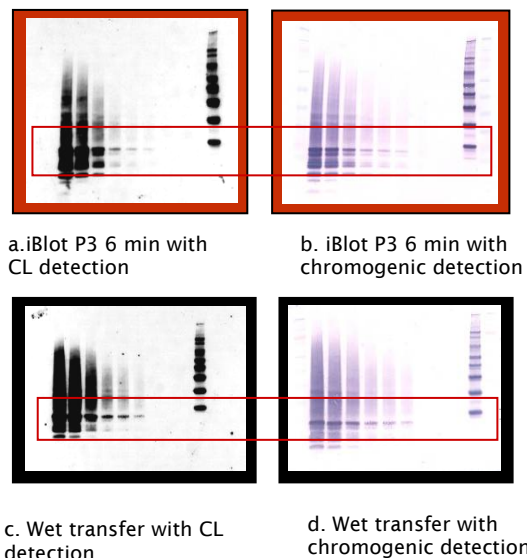


Figure 2. Membranes from NuPAGE® Novex® 4-12% Bis-Tris Gels with Histone purified protein (11-21 kDa) transferred with the iBlot™ Dry Blotting device (a,b) and an Invitrogen XCell II™ Blot Module (c,d).

Summary

Large and small protein transfers were optimized using the iBlot™ Dry Blotting System while providing overall visual immunodetection results that are comparable to or more sensitive than results obtained using a standard wet blotting system, but with just 6-8 minute transfer times.

When transferring large proteins with the iBlot™ system for NuPAGE® Novex® 4-12% Bis-Tris Gels, it is best to optimize the blot with the iBlot™ system and try NuPAGE® Novex® 3-8% Tris-Acetate Mini Gels along with adding more transfer time to the iBlot. Our data indicates that equilibration steps may not be needed with the Tris-Acetate gels since large proteins from these gels transfer more efficiently than from Bis-Tris gels. When using the P3 program, total transfer time for large proteins can be extended up to 10 minutes depending on the protein.

When transferring small proteins under 30 kDa with the iBlot™ Device, it is best to reduce the transfer time to less than 7 minutes to obtain the best results. In this experiment the P3 program for 6 minutes was used but optimization with less time can be tested with each individual protein.

For more information, visit www.invitrogen.com/iBlot.

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Product	Quantity	Cat. no.
NuPAGE® Novex® 4-12% Bis-Tris Gels, 1.0mm, 10 wells	box of 10	NP0321BOX
NuPAGE® Novex 3-8% Tris-Acetate Gel 1.0 mm, 12 well	box of 10	EA03752BOX
NuPAGE® MES Running Buffer 20X	500 ml	NP0002
NuPAGE® Tris-Acetate SDS Running Buffer (20X)	500 ml	LA0041
iBlot™ Gel Transfer Device	1 unit	IB1001
iBlot™ Transfer Stack, Regular (Nitrocellulose)	10 sets/box	IB301001
iBlot™ Transfer Stack, Mini (Nitrocellulose)	10 sets/box	IB301002
iBlot™ Transfer Stack, PVDF Regular	10 sets/box	IB401001
iBlot™ Transfer Stack, PVDF Mini	10 sets/box	IB401002
XCell II™ Blot Module	1 unit	EI9051
Nitrocellulose membranes, 0.2 µm for mini gels	20/box	LC2000
Nitrocellulose membranes, 0.45 µm for mini gels	20/box	LC2001
PVDF membranes, 0.2 µm for mini gels	20/box	LC2002
Invitrolon™ PVDF, 0.45 µm for mini gels	20/box	LC2005