Western blotting

Improving protein transfer with the iBlot 3 Western Blot Transfer System

Western blotting is one of the most common research techniques for the identification and quantitation of protein expression. However, western blotting is known to have several sources of variability that can influence the reliability of results. The transfer step, where proteins are electrophoretically transferred from a separation gel to a membrane, is one such source of variability, and performing high-efficiency protein transfers consistently is a common challenge among researchers. To address this challenge, many parameters must be considered, the most important of which is the transfer method.

The three most common protein transfer methods are wet, semi-dry, and dry transfer. The key differences between these methods are the amount of buffer used and the speed of the transfer. In traditional wet transfer systems, the filter paper–membrane–gel sandwich is submerged in a tank that contains transfer buffer. A current passes through the buffer to move proteins from the gel to the membrane. For semi-dry transfer, the membrane and gel are sandwiched between filter paper soaked with transfer buffer. This wetted filter paper serves as the buffer reservoir to support transfer. Charge is driven through the filter paper to move the proteins from the gel to the membrane. In dry transfer systems, the membrane–gel sandwich is placed between specialized gel matrices that contain ions. These ions move when current is applied, resulting in transfer of the proteins from the gel to the membrane. Both semi-dry and dry transfer offer significant transfer speed advantages over wet tank transfer.

See how dry transfer works with the iBlot 3 Western Blot Transfer System at thermofisher.com/iblot3

Historically, researchers struggled to find a commercially available semi-dry transfer system that consistently achieved higher transfer efficiency than wet transfer. This has led to the misconception that wet tank transfer offers the best transfer efficiency compared to semi-dry or dry transfer methods. For example, semi-dry and wet transfer methods were used to compare the transfer and detection of mTOR in a HEK293 lysate. Figure 1 shows that better transfer and detection was achieved by wet transfer, as demonstrated by stronger signals and higher sensitivity with a HEK293 dilution series. It should be noted that through optimizing the conditions of the semi-dry transfer method, it is possible to match or exceed the transfer performance of wet transfer; dry transfer is an alternative, even more convenient method to consider. Although there will certainly be cases where wet transfer could outperform the transfer efficiency of dry transfer, rapid dry transfer systems have been shown to provide excellent transfer efficiency compared to wet transfer [1]. Here we corroborate these reports and show that the Invitrogen™ iBlot™ 3 Western Blot Transfer System (dry transfer) routinely outperforms wet transfer methods.

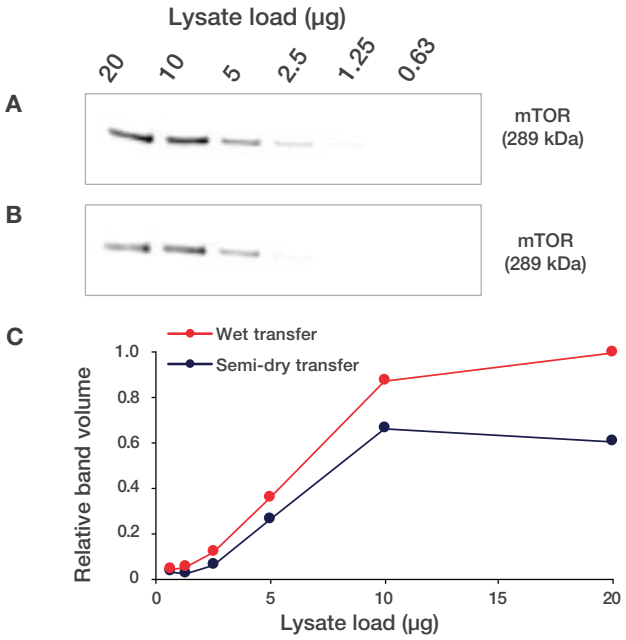

Figure 1. Chemiluminescence detection of mTOR in a dilution series of a HEK293 cell lysate, comparing semi-dry and wet transfer systems from Supplier B. Samples were separated using Invitrogen™ NuPAGE™ 3–8% Tris-Acetate Mini Protein Gels. Membrane images for **(A)** wet transfer (1 hr, 100 V) and **(B)** semi-dry transfer (10 min, 25 V). **(C)** Normalized band volumes for transfer and detection, comparing semi-dry and wet transfer systems.

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Another common misconception is that longer wet transfer times will improve transfer efficiency and subsequent immunodetection. Figure 2 illustrates how increased transfer time resulted in diminished signal and immunodetection of the protein Ku80. Of particular note, overnight transfer at a cold temperature (4°C) did not improve transfer efficiency and detection.

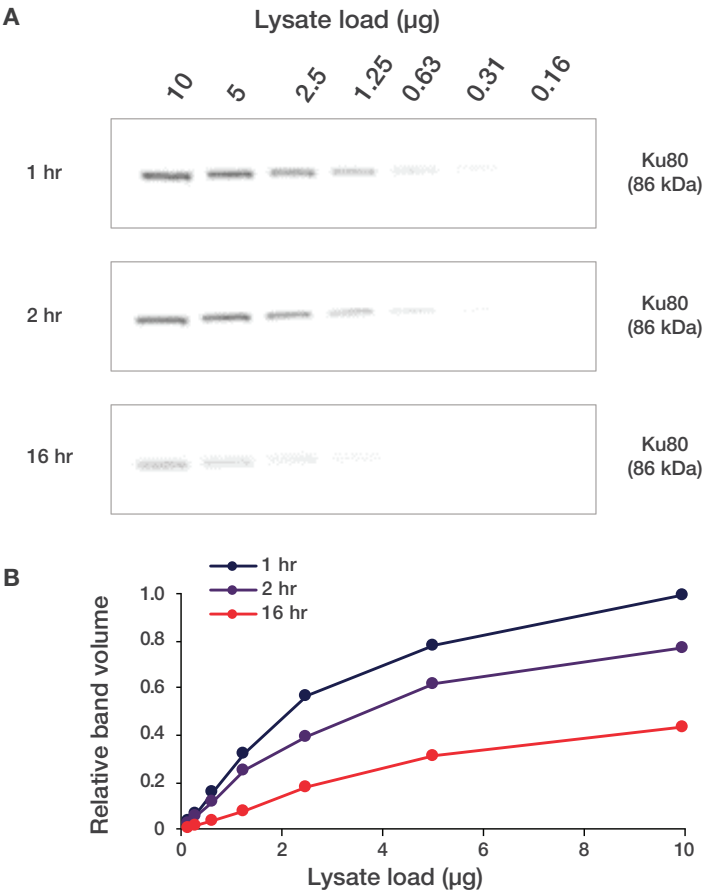

Figure 2. Chemiluminescence detection of Ku80 in a dilution series of a HEK293 cell lysate at increasing transfer times with a wet transfer system (1 or 2 hr, 100 V at room temperature; 16 hr, 30 V at 4°C). Samples were separated using Invitrogen™ Novex™ WedgeWell™ 4–20% Tris-Glycine Mini Protein Gels. **(A)** Membrane images for transfer times of 1, 2, and 16 hr. **(B)** Normalized band volumes for transfer and detection for transfer times of 1, 2, and 16 hr.

When optimized properly, wet transfer achieves good transfer efficiency, but this >40-year-old method of protein transfer [2] requires tedious preparation steps and long transfer times (≥1 hr). Researchers need a modern protein transfer solution that can deliver rapid results with equivalent or better transfer efficiency compared to wet transfer. Here we show this can be achieved by the Invitrogen™ iBlot™ 3 Western Blot Transfer System.

Methods

Electrophoresis was carried out as described in the user manuals for each gel type. Wet transfer was performed according to the manufacturer's instructions, using nitrocellulose membranes. For transfers on the iBlot 3 system, mini nitrocellulose transfer stacks were used and the voltage and transfer time for each experiment are noted in the figure legends. Immunodetection was completed using the Invitrogen™ Bandmate™ Automated Western Blot Processor. Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate was used for chemiluminescence detection. Imaging was performed using the Invitrogen™ iBright™ FL1500 Imaging System, and relative quantitation (band volume, which is band area multiplied by band intensity) was performed using iBright™ Analysis Software.

Summary of workflow improvements

The iBlot 3 Western Blot Transfer System reduces protein transfer time from ≥1 hour to 3–8 minutes, depending on the molecular weight of the target protein. For convenience, the iBlot 3 device is preprogrammed with three methods: high molecular weight, broad range, and low molecular weight, which are optimized to generate excellent protein transfer based on the molecular weight of the target proteins. Additionally, the iBlot 3 system provides rapid setup and reduced cleanup times compared to wet transfer. The preassembled iBlot 3 transfer stack is ready to use and does not require membrane activation or the preparation of transfer solution containing methanol, which requires hazardous material disposal. The stack is self-contained in its own tray for easy cleanup. Upon transfer completion, the tray is removed from the device and the cathode plate is wiped down to complete cleanup.

Transfer efficiency

In addition to the workflow improvements described above, the following data show that the iBlot 3 system also consistently achieves better transfer efficiency than wet transfer methods for several different proteins across a broad range of molecular weight and in three different cell lysates. Figure 3 shows a comparison between the iBlot 3 system (dry transfer) and wet transfer with subsequent detection of Ku80 expressed in HEK293 cells. With all other parameters (blocking, primary and secondary antibody incubations, and washes) held constant, dry transfer with the iBlot 3 system achieved superior transfer efficiency, as demonstrated by significantly higher chemiluminescence signal.

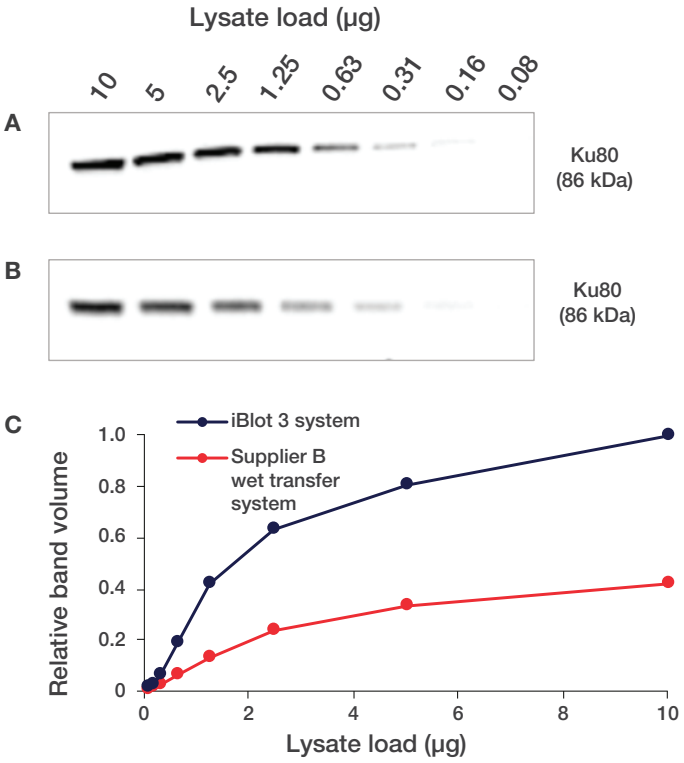

Figure 3. Chemiluminescence detection of Ku80 in a dilution series of a HEK293 cell lysate, comparing dry and wet transfer systems. Samples were separated using Novex WedgeWell 4–20% Tris-Glycine Mini Protein Gels. **(A)** Dry transfer with the iBlot 3 Western Blot Transfer System (6 min, 25 V, low cooling). **(B)** Wet transfer with the Supplier B wet transfer system (1 hr, 100 V). **(C)** Normalized band volumes for transfer and detection, comparing dry (iBlot 3 system) and wet (Supplier B) transfer systems.

In addition to Ku80, several other commonly studied protein targets were transferred and detected much more efficiently with dry transfer than with wet transfer of cell lysate dilution series (Figures 4–6). As shown in Figure 4, the high molecular weight protein mTOR transferred significantly better using the iBlot 3 system than with wet transfer. The preprogrammed high molecular weight method was used to maximize transfer in only 8 minutes compared to the 1 hour wet transfer.

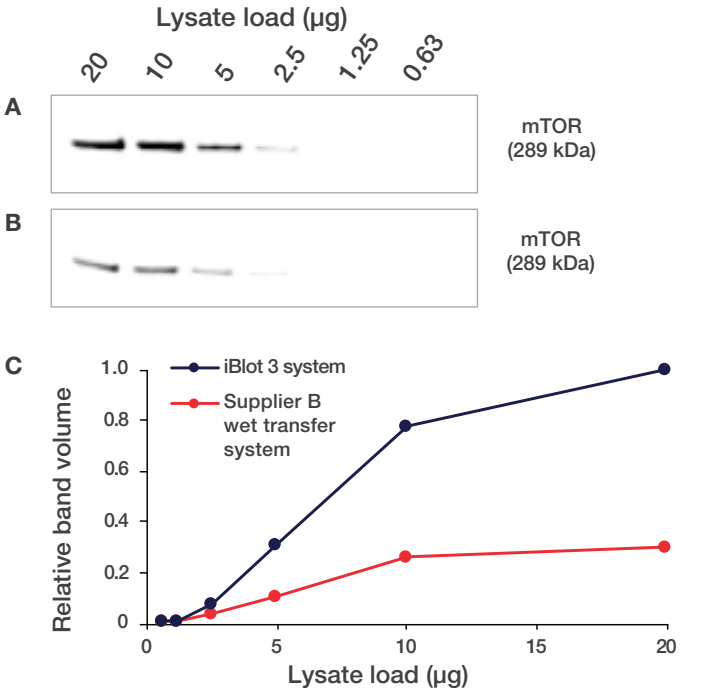

Figure 4. Chemiluminescence detection of mTOR in a dilution series of a HEK293 cell lysate, comparing dry and wet transfer systems. Samples were separated using NuPAGE 3–8% Tris Acetate Mini Protein Gels. **(A)** Dry transfer with the iBlot 3 Western Blot Transfer System (8 min, 30 V, no cooling). **(B)** Wet transfer with the Supplier B wet transfer system (1 hr, 100 V). **(C)** Normalized band volumes for transfer and detection, comparing dry (iBlot 3 system) and wet (Supplier B) transfer systems.

Figures 5 and 6 illustrate the transfer and detection of two different medium molecular weight proteins, PDI and Hsp70, in HepG2 and A431 cell lysates, respectively. In both cases transfer efficiency was higher with the 6 minute preprogrammed broad range transfer method of the iBlot 3 system than with the 1 hour wet transfer with the Supplier B wet transfer system.

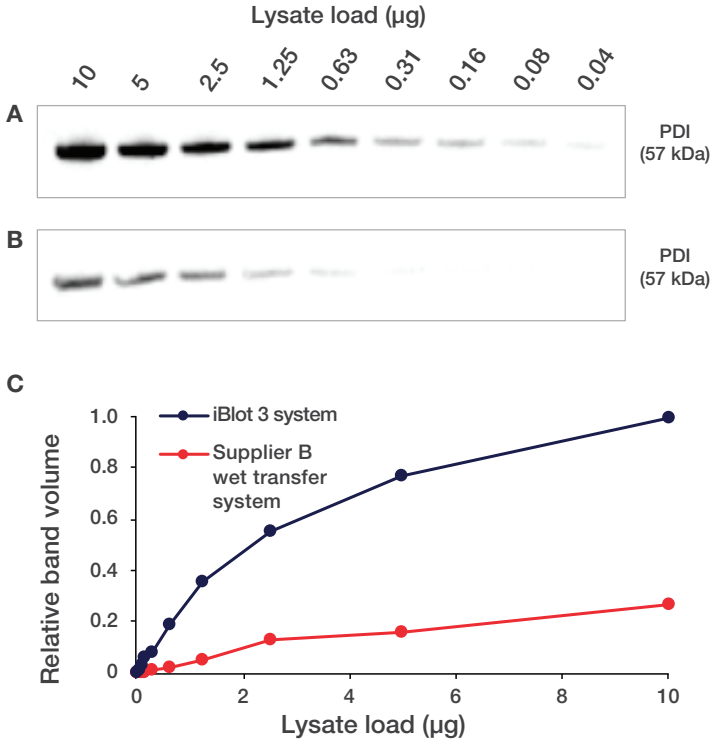

Figure 5. Chemiluminescence detection of PDI in a dilution series of a HepG2 cell lysate, comparing dry and wet transfer systems. Samples were separated using NuPAGE 4–12% Bis-Tris gels. **(A)** Dry transfer with the iBlot 3 Western Blot Transfer System (6 min, 25 V, low cooling). **(B)** Wet transfer with the Supplier B wet transfer system (1 hr, 100 V). **(C)** Normalized band volumes for transfer and detection, comparing dry (iBlot 3 system) and wet (Supplier B) transfer systems.

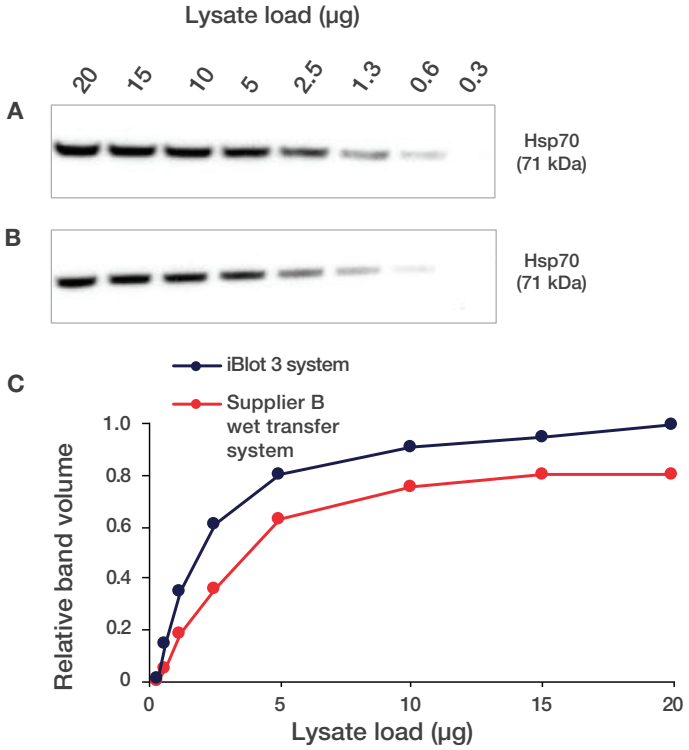

Figure 6. Chemiluminescence detection of Hsp70 in a dilution series of an A431 cell lysate, comparing dry and wet transfer systems. Samples were separated using NuPAGE 4–12% Bis-Tris gels. **(A)** Dry transfer with the iBlot 3 Western Blot Transfer System (6 min, 25 V, low cooling). **(B)** Wet transfer with the Supplier B wet transfer system (1 hr, 100 V). **(C)** Normalized band volumes for transfer and detection, comparing dry (iBlot 3 system) and wet (Supplier B) transfer systems.

Summary

This study highlighted the benefits of western blot dry protein transfer over traditional wet tank transfer. The iBlot 3 Western Blot Transfer System is a convenient alternative to wet tank transfer, and as shown in this study it helps enable improved transfer efficiency and immunodetection, as demonstrated by improved sensitivity and signal intensity. The iBlot 3 dry transfer system is also significantly faster than wet transfer and more effectively transfers high- and medium-range molecular weight proteins in 6–8 minutes, compared to a 1 hour wet transfer. Furthermore, the quick setup and easy cleanup of the iBlot 3 dry transfer system differentiate it from wet transfer as a more modern and effective protein transfer solution.

References

1. Silva JM, McMahon M (2014) The fastest western in town: a contemporary twist on the classic western blot analysis. *J Vis Exp.* 84:e51149. doi:10.3791/51149
2. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A.* 76(9):4350-4354. doi:10.1073/pnas.76.9.4350

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