Western blotting

Automated Western Blot Processing with the iBind™ Western Devices

Introduction

Western blotting is an important research tool for monitoring the regulation of proteins in core applications across cancer, immunology, infectious disease, and neurobiology as well as growing areas such as antibody-drug conjugate (ADC) therapeutics and cell and gene therapy. While widely adopted, the western blot processing procedure can be time-consuming and labor-intensive involving multiple rounds of blocking, washing and, extended antibody incubations. Because of this it can be difficult to multitask or conduct other experiments while processing a western blot, especially when multiple blots are being processed simultaneously. In addition, the number of touch points involved in a typical western blot experiment, can increase the chance of errors or increase variability.

Due to the number steps in a western blot experiment, efficiently automating the western blot process has proven to be difficult or expensive. The iBind Western Devices and iBind PLUS reagent kits offer a simple, and convenient method for streamlining the manual steps in western blot processing while maintaining and even improving the signal quality during final detection. The iBind western blot system completes the western processing method in 2.5-3 hours with only one initial touchpoint compared to manual blocking, washing and immunoprobing requiring 4-24 hours with over 10 touchpoints (Figure 1). iBind reagents are loaded in the device at the start of the procedure to subsequently enable uninterrupted and hands-free blocking, antibody incubation, and washing steps through simple capillary action based sequential lateral flow technology.

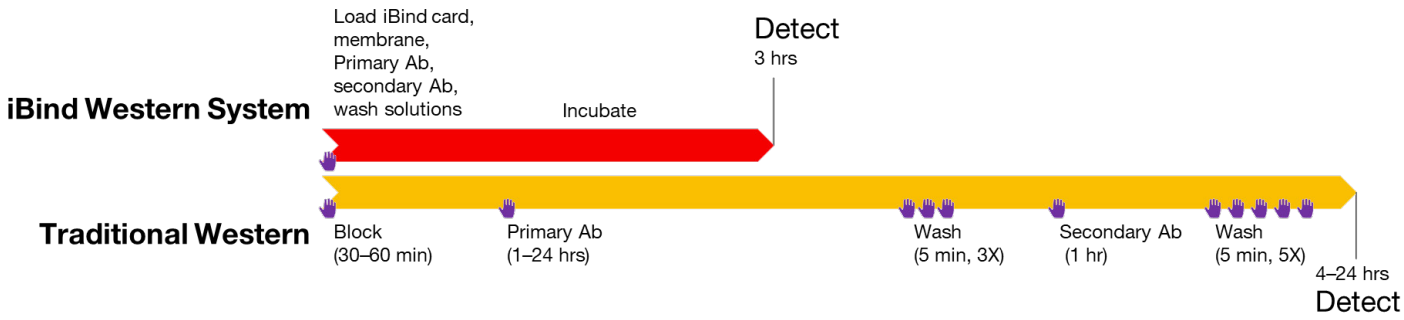


Figure 1. iBind Western Systems reduce hands-on time and streamline western blot processing. Comparison of a traditional western blot workflow (yellow) and the iBind workflow (red). Hand icons indicate user touch points. Traditional workflows require multiple manual incubation and wash steps throughout the assay, resulting in frequent hands-on interaction. In contrast, the iBind system automates these steps using a sequential lateral flow process, reducing hands-on time to a single setup step while the assay proceeds unattended.

How it works

iBind Western Systems are simple devices that utilize sequential lateral flow (SLF) to move immunodetection reagents automatically and sequentially through the iBind Card and across the membrane surface without the need of electricity or mechanical action. Each buffer reagent flows through the card sequentially, following the previous reagent. Little to no mixing of reagents is seen and each solution flushes the previous solution off the membrane (Figure 2). The use of lateral flow means only a thin layer of liquid is constantly flowing across the membrane.

This thin layer makes it possible to use small volumes of antibody and wash solutions (2ml) without risk of the membrane drying out or abnormal coverage due to shaking or rocking. In addition, antibodies and buffers are always a minimal distance away from the membrane and fresh reagent is consistently replacing spent reagent, thereby minimizing diffusion distances and interference, which increases antibody binding.

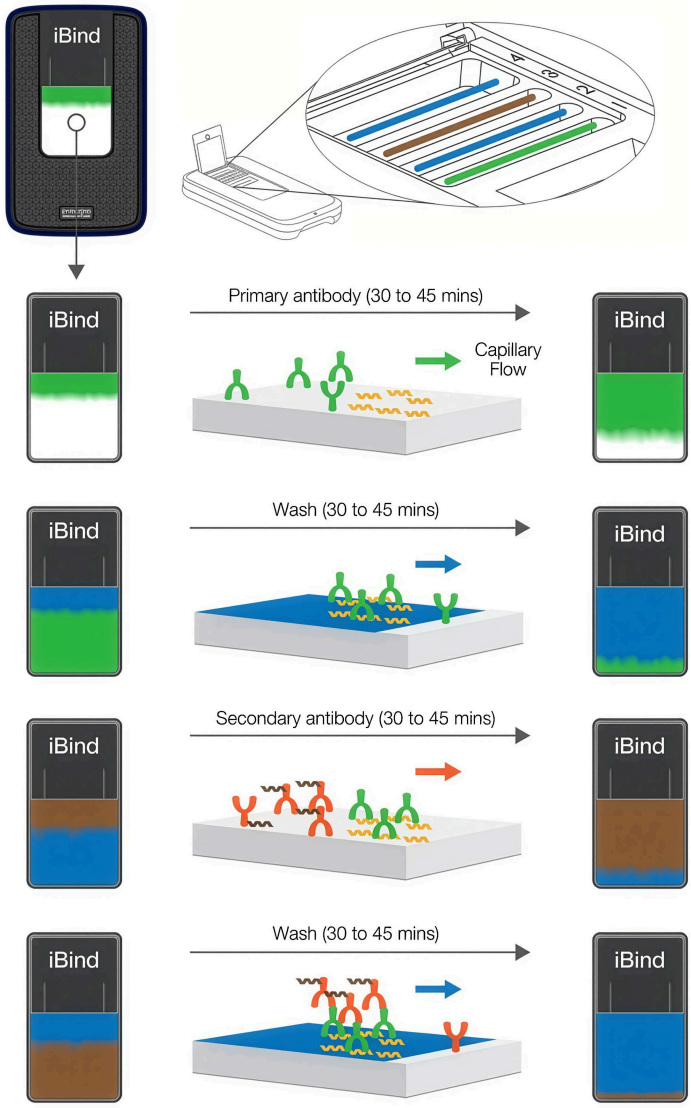


Figure 2. Sequential lateral flow–driven reagent delivery in the iBind system. The iBind device passively transports reagents across the membrane using sequential lateral flow through the iBind card. Distinct colored dyes were loaded into individual wells to visualize flow dynamics over time. Each reagent is released in a controlled sequence, forming discrete fronts that migrate across the iBind card via capillary action. Minimal mixing between adjacent fronts is observed, demonstrating efficient sequential delivery of primary antibody, wash, secondary antibody, and final wash steps without the need for external pumps or user intervention.

Improving reproducibility while reducing antibody usage

The time and labor savings of the iBind system can greatly improve productivity, but just as importantly iBind PLUS reagents also can significantly improve western blot sensitivity while reducing antibody costs.

In 2026, the iBind PLUS reagent kits were introduced to replace the original iBind reagent kits. The iBind PLUS kits enhance the iBind workflow in terms of ease of use, sensitivity and antibody utilization. The iBind PLUS Solution Kit and iBind Flex PLUS Solution Kit are designed for chemiluminescent detection, and each kit contains two ready-to-use components: the iBind PLUS Solution and iBind PLUS Antibody Diluent. These ready-to-use 1X formulations replace the original kit's 5X concentrate, which required dilution and addition of additives before each step. The iBind PLUS reagents offer a greater level of convenience and improve performance as each solution has been optimized for its specific function in sequential lateral flow processing. The iBind PLUS Solution is used directly in the membrane blocking, card wetting, and washing steps. It shares a similar composition to the original iBind solution, but the blocking strength has been optimized. There is no need to purchase additional blockers and buffers that are typically used in traditional western blotting.

The iBind PLUS Antibody Diluent is used to dilute the primary and secondary detection antibodies, and is designed to maximize western blot signal within the short processing time of the iBind workflow. The Diluent enhances antibody binding to the target of interest by 3 mechanisms: reducing interference from the blocking buffer, improved antibody solubility, and the addition of bulking reagents to enhance antibody binding to the target protein.

This means that for many targets, when western blots processed with the iBind systems and iBind PLUS reagents are compared to the original iBind kits or to western blots processed manually with a 16-hour overnight incubation in primary antibody, significantly more signal is seen for the iBind PLUS processed samples despite less primary being used. Figure 3 compares western blot results of the original iBind Reagents to the iBind PLUS reagents under a variety of primary antibody concentrations. Use of the iBind PLUS reagents resulted in significantly reduced exposure times and improved limits of detection under all conditions. This means significantly lower antibody concentrations can be used with the iBind PLUS reagents while still achieving high quality western blot results. In the example provided in Figure 3, usage of four-fold less primary antibody with iBind PLUS still results in significantly more signal generation than the original iBind method, as seen by the less than 8 times lower exposure time requirements (9s vs 71s).

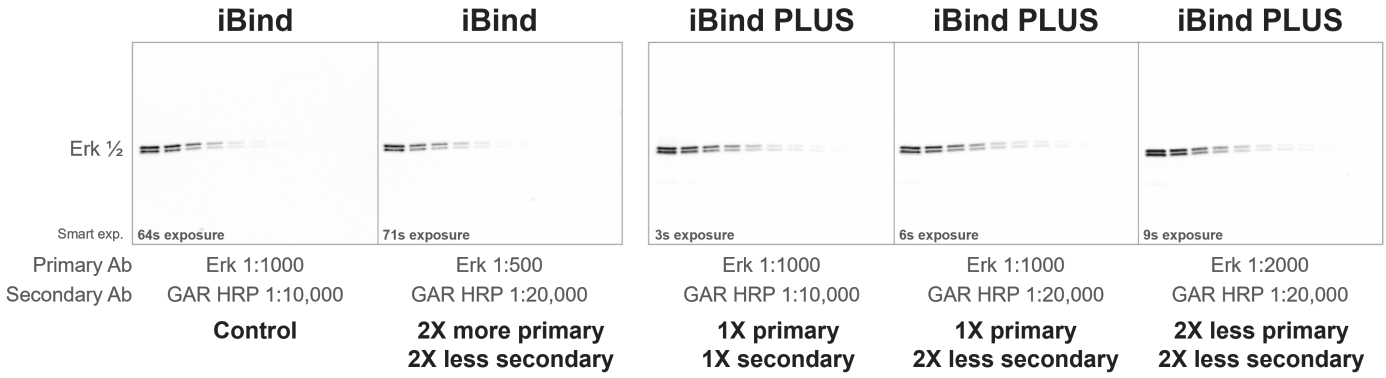


Figure 3. The iBind PLUS reagents allow primary antibody concentrations to be significantly reduced while providing increased signal sensitivity compared to the original iBind solutions. Comparison of western blot band intensities at different detection antibody dilutions, after processing with the iBind System using either the original iBind Reagents or the iBind PLUS reagents. For each experimental condition, a dilution series (2-Fold) of cell lysate (5µg lysate start) was separated and then transferred to nitrocellulose membranes using Invitrogen iBlot 3 transfer system. The blots were then separated to perform immunoprocessing using either the classic iBind reagents or iBind PLUS Solution Kit. Blots were processed with 2mL of primary antibody and 2mL of secondary-HRP antibody at differing dilution amounts as indicated in the figure. For final detection, blots were incubated for 5 minutes in Thermo Scientific™ SuperSignal™ West Dura Substrate and then imaged under optimal smart exposure times for each membrane and condition.

Figure 4 compares the western blot results with 3-hour iBind Device sequential lateral flow processing with iBind PLUS reagents to manually processing blots with a 16-hour overnight incubation in primary antibody, for three targets under a variety of primary antibody concentrations. For some targets, equal or better detection can be achieved with iBind processing even when using 25-fold less primary antibody compared to overnight manual immunoprobing. This reduction in antibody usage is due to a combination of Antibody volume used (2mL instead of 10 mL) and concentration.

Figure 5 demonstrates the performance advantage of iBind PLUS reagents across a panel of primary antibodies imaged using manual exposures for comparison across all processing methods. The iBind PLUS reagents significantly improve results for most targets allowing users to minimize exposure times, increase the limit of detection, or significantly reduce primary antibody usage compared to both the original iBind method and manually processed western blots.

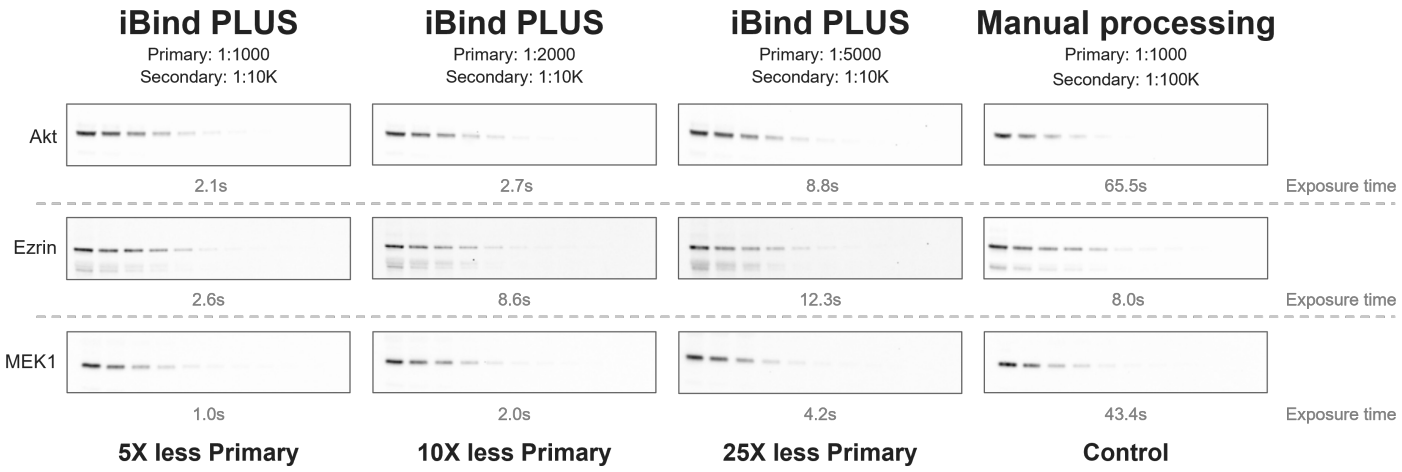


Figure 4. Primary antibody consumption can be reduced to up to 25X using iBind Western Systems while maintaining similar sensitivity compared to traditional processing. iBind Western Systems with iBind PLUS Solution Kits enable reduction of primary antibody consumption while maintaining strong signal detection compared to manual western blot processing. A431 lysate starting at 10 µg was two-fold serial diluted, separated on Novex 4–20% Tris-Glycine gels (Cat No. XP04202BOX), and transferred on Nitrocellulose membranes using the iBlot 3 Western Blot Transfer System (Cat No. IB31001 and IB33002). Membranes were then processed with primary antibodies for 3 targets (AKT, Ezrin, and MEK1) using AKT Pan Monoclonal Antibody (Cat No. MA5-14916), Ezrin Monoclonal Antibody (Cat No. 35-7300), or MEK1 Monoclonal Antibody (Cat No. 13-3500), respectively, at varying concentrations as indicated, followed by incubation with secondary-HRP antibody diluted at 1:10,000 as recommended for the iBind Western System protocol. Manual western blotting was processed using Pierce Clear Milk Blocking Buffer (Cat No. 37587) for 1 hour, followed by the same primary antibodies as used in iBind western system for each respective target at a 1:1000 in Pierce Clear Milk Blocking Buffer and incubated overnight at 4°C (18 hours). Secondary-HRP antibody was diluted 1:100,000 for each blot. All blots were incubated with SuperSignal™ West Dura substrate and imaged under optimal exposure times for each blot using the iBright FL1500 Imaging System.

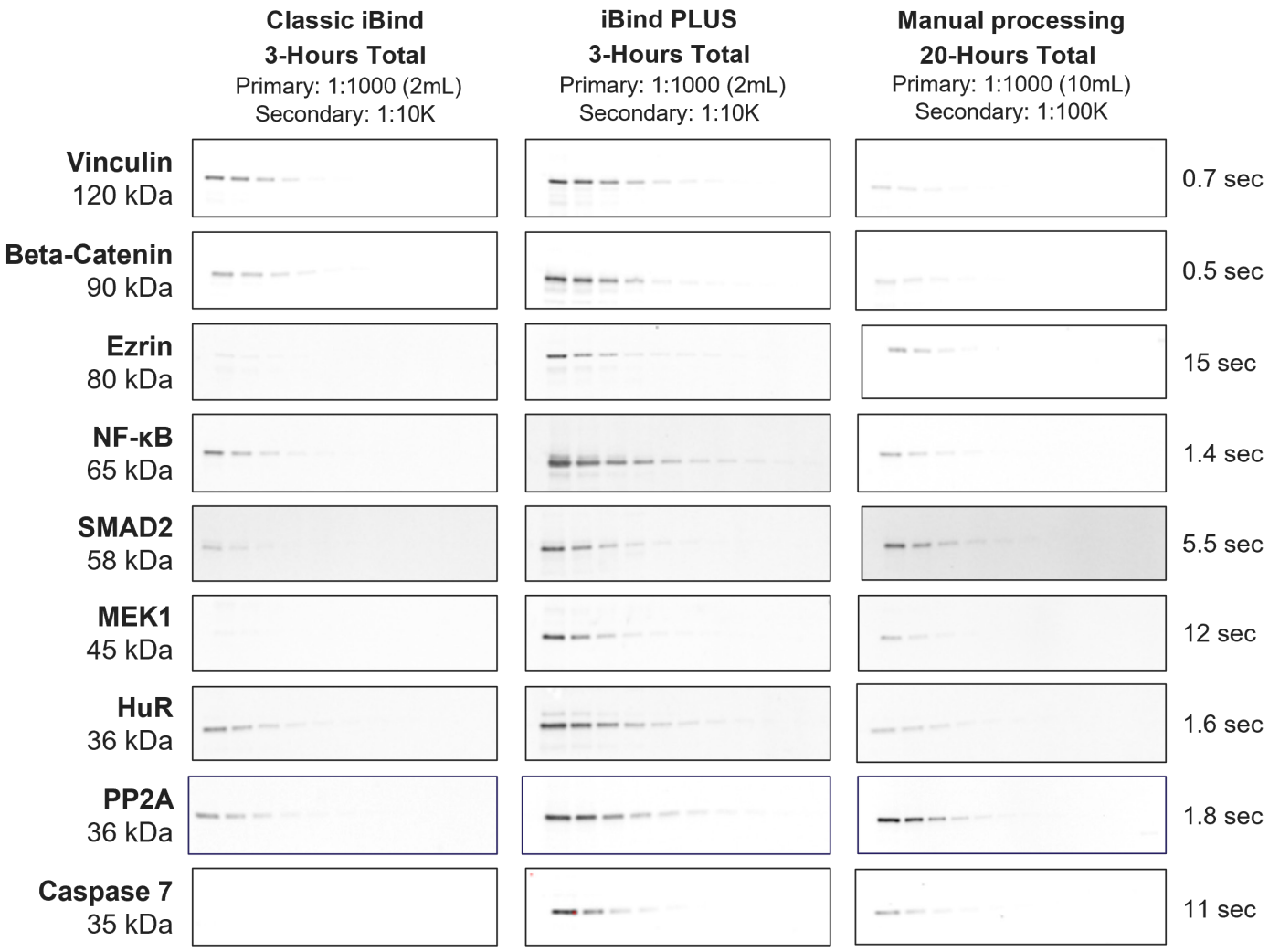


Figure 5. Comparison of western blot band intensities for multiple different targets using the same manual exposure time, after processing with the original iBind Reagents, the iBind PLUS reagents, or manual western blot processing. For each target, a dilution series (2-Fold) of cell lysate (5µg lysate start) was separated and then transferred to nitrocellulose membranes using Invitrogen iBlot 3 transfer system. The blots were then separated to perform immunoprocessing using either the classic iBind reagents (left side) or iBind PLUS Solution Kit (Middle) or processed manually by hand. Blots processed with iBind devices used 2mL of primary antibody (diluted 1:1,000) and 2mL of secondary-HRP antibody (diluted 1:10,000). Manually processed blots were immunoprobed in 10ml of primary antibody diluted 1:1000 in blocker for 16-hours with rocking, followed by washing with TBST (3x10ml for 5 minutes each. The blot was then incubated in 10mL of 1:100,000 HRP-labeled secondary antibody (SKU) for 1-hour followed by final washing in TBST (5x10ml for 5 minutes each). For final detection, blots were incubated for 5 minutes in Thermo Scientific SuperSignal West Substrates (Dura or Atto) and then imaged under the same exposure times on an iBright Imaging system for direct visual comparison of intensity differences. Each blot produced clean and clear result when imaged individually, at a time appropriate for each sample (data not shown).

The iBind Western System is offered in two formats – iBind and iBind Flex. Both devices are compact and operate without any electricity, which the various sizes allowing for greater flexibility and making converting from standard western blot processing easy. The iBind device processes one mini membrane while the iBind Flex system includes three interchangeable inserts for processing 2 mini blots, one midi blot, or up to six vertically cut membrane strips at a time.

Both iBind Western Systems require no electricity or batteries, making them a more sustainable option for western blot workflows. By combining the fast protein separation of Invitrogen Bolt Bis-Tris Plus SDS-PAGE gels (15 minutes with MES running buffer at 250V), the efficient 6-minute transfer of iBlot3, the hands-free 2.5-3 hour processing of iBind System, and the smart imaging capture using iBright Imagers, high quality western blots can easily and reliably be completed and analyzed in less than 4 hours (Figure 6.)

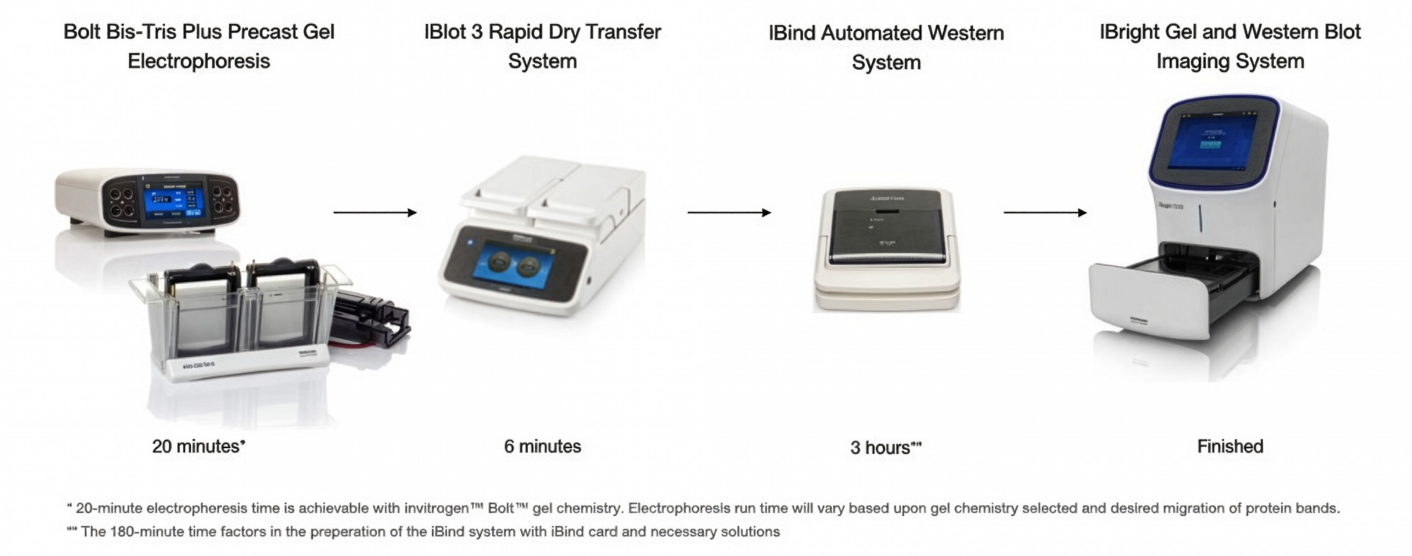


Figure 6. Streamlined western blot workflow using Invitrogen systems. Fast protein separation with Bolt™ Bis-Tris Plus gels (20 minutes), rapid transfer with iBlot™ 3 (6 minutes), hands-free processing with the electricity-free iBind™ Western System (~3 hours), and imaging with iBright™ Imaging Systems enable high-quality western blot results in under 4 hours.

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