Revolutionary western blot processing—no shakers, trays, or timers required

Automated iBind Western Systems

Invitrogen™ iBind™ Western Systems are automated western blot processing platforms. Simply load primary antibody, secondary antibody, and wash solutions, and then walk away. In less than three hours, the blot is ready for final detection. iBind Western Systems offer:

- **Flexibility**—pick the system that matches your throughput; process 1 mini or midi blot, 2 mini blots, or 6 vertically cut strips using the same or different conditions
- **Antibody savings**—use up to 80% less primary antibody
- **Load and go**—the system processes solutions using sequential lateral flow technology, with no batteries, shakers, trays, or timers required

iBind Western Starter Kit (Cat. No. SLF1000S)

- **Reproducibility**—automated blot processing enables improved blot-to-blot consistency

	iBind Western Device	iBind Flex Western Device
		
Mini blot (8 x 8 cm), single	Yes	Yes
Mini blot (8 x 8 cm), dual	No	Yes
Midi blot (13 x 8 cm)	No	Yes
Vertically cut strips (up to 6)	No	Yes

Watch a video demonstration at **thermofisher.com/ibind**

iBind Flex Western System

The Invitrogen™ iBind™ Flex Western System offers flexible blot processing to optimize antibody use, easily change blot formats, and reduce hands-on time. Simply load primary and secondary antibody solutions and wash solutions into the device, then walk away, because the system automatically performs all immunodetection steps using SLF technology, a simple form of capillary action. In less than three hours, the blot is ready for final detection. The iBind Flex Western System delivers flexible solutions with automated convenience.

Features:

- **Flexibility** — process up to one midi blot, two mini blots or six vertically cut strips using the same or different conditions
- **Compatibility** — use nitrocellulose or PVDF membranes, directly labeled primary or secondary antibody detection (AP, HRP or fluorescent labeled)
- **Cost savings** — use up to 80% less primary antibody than with traditional tray-based incubation steps for Western blotting

The iBind Flex Western System comes with interchangable wells, which allow you to run multiple membrane formats and even run different primary and secondary antibody conditions in the same device at the same time.

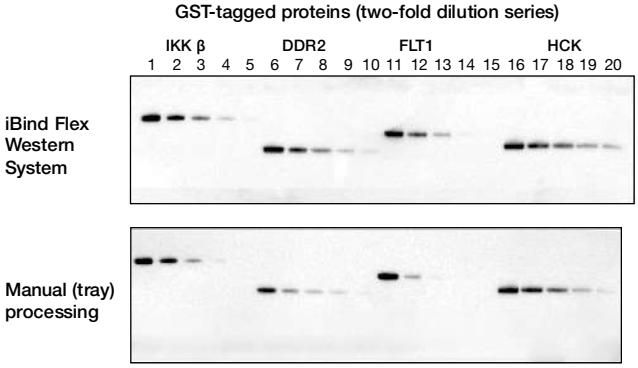

Figure 4. Comparison of Western blots processed manually vs. with the iBind Flex Western System. Samples containing GST-tagged recombinant proteins were separated on Invitrogen™ NuPAGE™ 4–12%, 20-well midi gels in MOPS SDS running buffer and then transferred to nitrocellulose membranes using the Invitrogen™ iBlot™ Dry Blotting System. Blots were probed with identical concentrations of the same pair of primary and secondary antibodies. The primary antibody was rabbit anti-GST diluted 1:500 (8µL in 4mL iBind Flex Solution for the iBind system, 40µL in 20mL for manual tray incubation). The secondary antibody was goat anti-rabbit HRP diluted 1:600 (6.7µL in 4mL iBind Flex Solution for the iBind system, 33.3µL in 20mL for manual tray incubation). For final detection, blots were incubated for five minutes in SuperSignal West Dura Extended Duration Substrate for visualization with an imaging system.
Lanes 1–5: IKK β (80ng, 40ng, 20ng, 10ng, 5ng)
Lanes 6–10: DDR2 (120ng, 60ng, 30ng, 15ng, 7.5ng)
Lanes 11–15: FLT1 (40ng, 20ng, 10ng, 5ng, 2.5ng)
Lanes 16–20: HCK (360ng, 180ng, 90ng, 45ng, 22.5ng)

Midi blots	Mini blots	Vertically cut strips
		
Using same antibody conditions	Using same or different antibody conditions	Using same or different antibody conditions

For ordering information refer to page 78.

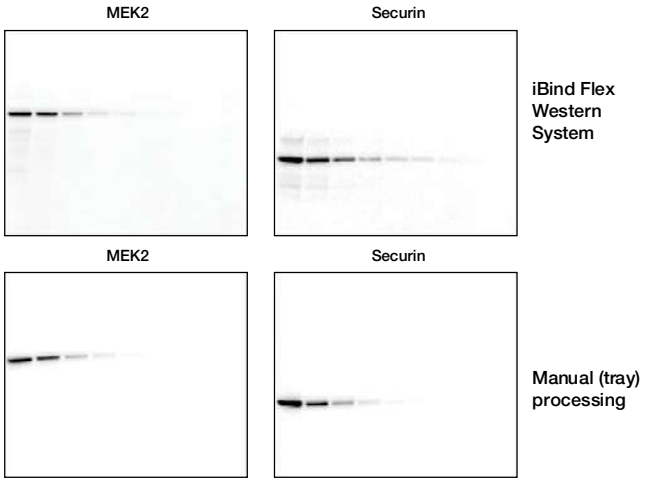

Figure 5. Better Western blot results using less primary antibody. Comparison of mini blots processed manually (probing and washing steps performed in a tray) vs. with the iBind Flex Western Device. Blots were produced by separating samples on Invitrogen™ Bolt™ 4–12% Bis-Tris 10-well mini gels with MES SDS running buffer and then transferred to nitrocellulose membrane using the Invitrogen™ iBlot™ device. Each gel contained 10 lanes loaded with two-fold dilution series of 293 cell extracts (30µg to 0.06µg). After immunodetection using the conditions described below, blots were incubated for five minutes in SuperSignal West Dura substrate and the signal documented with an imager.

- MEK2 (45kDa): rabbit anti-MEK2 antibody applied at 1:1,000 for iBind blot processing (2µL in 2mL of iBind Flex Solution), and 1:1,000 for manual blot processing (10µL in 10mL buffer).
- Securin (428kDa): rabbit anti-Securin antibody applied at 1:1,000 for iBind blot processing (2µL in 2mL of iBind Flex Solution), and 1:1,000 for manual blot processing (10µL in 10mL buffer).
- Secondary Antibody (both targets): goat anti-rabbit IgG at 1:600 for iBind blot processing (3.33µL in 2mL of iBind Flex Solution), and at 1:1,800 for manual blot processing (5.55µL in 10mL).

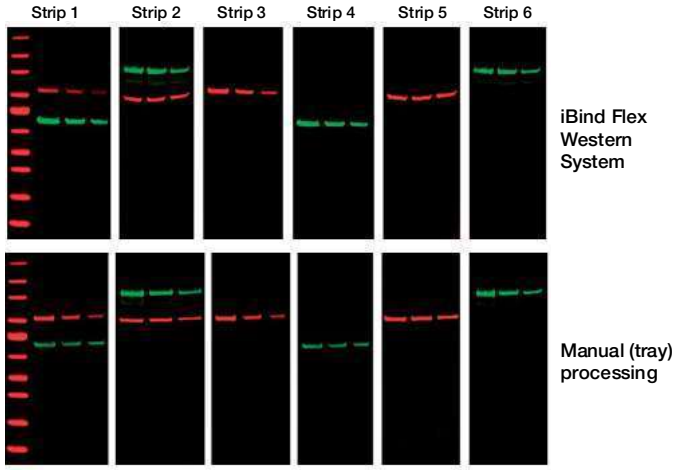

Figure 6. Excellent Western blot results with vertically cut strips and fluorescence detection. Comparison of mini blots processed manually (probing and washing steps performed in a tray) vs. with the iBind Flex Western Device. Blots were produced by separating samples on Bolt Bis-Tris Plus 4–12% 10-well mini gels with MES SDS running buffer. They were then transferred to nitrocellulose membranes using the iBlot 2 Gel Transfer Device, cutting each into three-lane strips. Final imaging was performed using the Odyssey™ CLx Infrared Imaging System.
Samples and lanes were as follows:

- Lane 1 Strip 1: Thermo Scientific™ PageRuler™ Prestained NIR Protein Ladder (3µL)
- Strip 1: Phosphorylated AKT cell extract (15µg, 7.5µg, 3.75µg) and Elk-1 Fusion Protein (150ng, 75ng, 37.5ng)
- Strip 2: HeLa cell extract (30µg, 15µg, 7.5µg)
- Strip 3: Phosphorylated AKT cell extract (15µg, 7.5µg, 3.75µg)
- Strip 4: Elk-1 fusion protein (150ng, 75ng, 37.5ng)
- Strip 5: HeLa cell extract (30µg, 15µg, 7.5µg)
- Strip 6: HeLa cell extract (30µg, 15µg, 7.5µg)

Probing and antibody conditions using the iBind Flex FD Solution were as follows:

Strip #	1° Ab	[1° Ab] Manual	[1° Ab] iBind Flex	2° Ab	[2° Ab] Manual	[2° Ab] iBind Flex
1	Phospho-AKT (red) Phospho-Elk-1 (green)	1:2,000 1:1,000	1:400 1:200	IRDye 680LT IRDye 800CW	1:20,000 1:15,000	1:4,000 1:3,000
2	SRC (red) beta-Catenin (green)	1:250 1:1,000	1:50 1:200	IRDye 680LT IRDye 800CW	1:20,000 1:15,000	1:4,000 1:3,000
3	Phospho-AKT	1:2,000	1:400	IRDye 680LT	1:20,000	1:4,000
4	Phospho-Elk-1	1:1,000	1:200	IRDye 800CW	1:15,000	1:3,000
5	SRC	1:250	1:50	IRDye 680LT	1:20,000	1:4,000
6	beta-Catenin	1:1,000	1:200	IRDye 800CW	1:15,000	1:3,000

After completion of Western processing, blots were imaged on the Odyssey CLx instrument.

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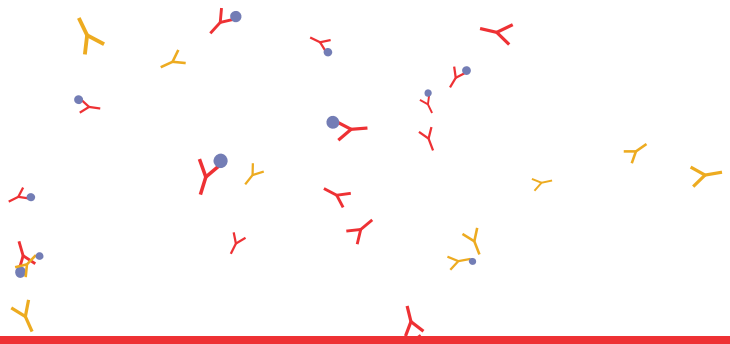

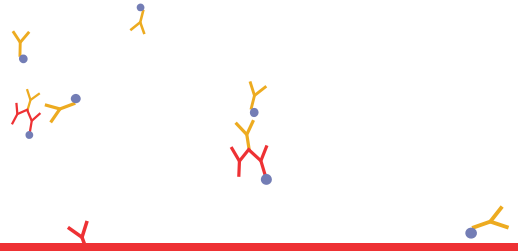

iBind Western System

The Invitrogen™ iBind™ Western System for mini blots is an automated Western blot processing platform that requires less primary antibody and enables sensitive, reproducible Western results. All blocking, antibody incubation and washing steps are hands-free, allowing you to load your solutions and walk away. No electricity or battery is required. You can also use your existing chemiluminescent, chromogenic or fluorescent Western detection protocols, including primary or secondary antibody conjugates of horseradish peroxidase (HRP), alkaline phosphatase (AP) or fluorophores. Automated processing enables improved blot-to-blot consistency with coefficients of variation (CVs) typically less than 5% (compared to manual processing that can have CVs of 13%).

Features:

- **Cost savings** — use up to 80% less primary antibody than with traditional tray-based incubation steps for Western blotting
- **Reproducibility** — automated immunodetection enables improved blot-to-blot consistency
- **Simplicity** — load solutions and walk away using SLF technology; no batteries or electricity required

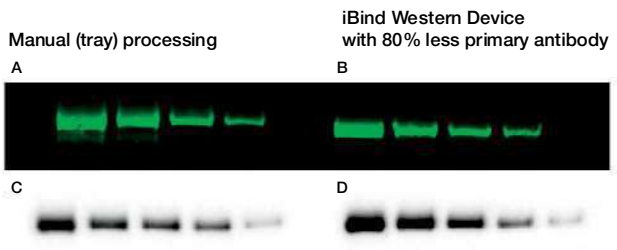

Figure 7. Using chemiluminescent or fluorescence-based detection methods, you can use up to 80% less primary antibody than manual methods. A 2-fold dilution series of EGF receptor control cell lysate (30µg, 15µg, 7.5µg, 3.75µg and 1.875µg) was used. Proteins were separated using the Bolt Bis-Tris Plus mini gels and transferred to PVDF membranes using the iBlot Dry Blotting System. The blots were probed with a mouse anti–phospho-EGF receptor [Tyr1068] (1H12) antibody (1:1,000 dilution, equated to 2µL antibody for the iBind device method and 10µL antibody for the manual method) followed by a goat anti–mouse IgG (H+L) peroxidase-conjugated antibody (1:360 for iBind device processing (5.55µL); 1:1,800 for manual method (5.55µL)). The standard iBind Solution Kit was used for the chemiluminescence blot (panel C, manual processing and panel D, iBind system processing); the iBind FD Solution Kit was used for fluorescence detection (panel A, manual processing and panel B, iBind processing). These results demonstrate that Western blots processed on the iBind device show comparable results to those obtained when Western blots are processed manually, with lower overall primary antibody requirements for the iBind device.

Learn more at **thermofisher.com/ibind**

For ordering information refer to page 78.

Ordering information

Product	Quantity	Cat. No.
Block, wash, probe		
Automated		
iBind Flex Western Starter Kit	1 kit	SLF2000S
iBind Flex Western Device	1 device	SLF2000
iBind Flex Cards	10 cards	SLF2010
iBind Flex FD Solution Kit	1 kit	SLF2019
iBind Flex Solution Kit	1 kit	SLF2020
iBind Western Starter Kit	1 kit	SLF1000S
iBind Western Device	1 device	SLF1000
iBind Cards	10 cards	SLF1010
iBind FD Solution Kit	1 kit	SLF1019
iBind Solution Kit	1 kit	SLF1020
Blocking solutions		
Membrane Blocking Solution	1L	00-0105
WesternBreeze Blocker/Diluent (Part A and B)	80mL	WB7050
Blocker BLOTTO in TBS	1L	37530
Blocker BSA in PBS (10X)	200mL	37525
Blocker BSA in TBS (10X)	125mL	37520
Blocker Casein in PBS	1L	37528
Blocker Casein in PBS	100mL	37582
Blocker Casein in TBS	1L	37532
Pierce Clear Milk Blocking Buffer (10X)	100mL	37587
Pierce Fast Blocking Buffer	500mL	37575
Pierce Fast Blocking Buffer	100mL	37576
Blocker Casein in TBS	100mL	37583
Pierce Protein-Free (PBS) Blocking Buffer	1L	37572
Pierce Protein-Free (PBS) Blocking Buffer	100mL	37584

Product	Quantity	Cat. No.
Pierce Protein-Free (TBS) Blocking Buffer	1L	37570
Pierce Protein-Free (TBS) Blocking Buffer	100mL	37585
Pierce Protein-Free T20 (PBS) Blocking Buffer	1L	37573
Pierce Protein-Free T20 (TBS) Blocking Buffer	1L	37571
SEA BLOCK Blocking Buffer	500mL	37527
SEA BLOCK Blocking Buffer	3 x 500mL	37527X3
StartingBlock (PBS) Blocking Buffer	1L	37538
StartingBlock (PBS) Blocking Buffer	100mL	37578
StartingBlock (TBS) Blocking Buffer	1L	37542
StartingBlock (TBS) Blocking Buffer	100mL	37579
StartingBlock T20 (PBS) Blocking Buffer	1L	37539
StartingBlock T20 (TBS) Blocking Buffer	1L	37543
SuperBlock (PBS) Blocking Buffer	1L	37515
SuperBlock (PBS) Blocking Buffer	5L	37518
SuperBlock (PBS) Blocking Buffer	100mL	37580
SuperBlock (PBS) Blocking Buffer - Blotting	1L	37517
SuperBlock (TBS) Blocking Buffer	1L	37535
SuperBlock (TBS) Blocking Buffer	100mL	37581
SuperBlock (TBS) Blocking Buffer - Blotting	1L	37537
SuperBlock (TBS) Blocking Buffer Dry Blend	5 packs	37545
SuperBlock T20 (PBS) Blocking Buffer	1L	37516
SuperBlock T20 (TBS) Blocking Buffer	1L	37536
I-Block Protein-Based Blocking Reagent	30g	T2015