

iBind™ Western System

Catalog Number SLF1000

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Instructions for using the iBind™ Western Device in a western blot workflow are described below. For detailed instructions and guidance on optimizing results, refer to the user manual available from thermofisher.com/ibind.

General guidelines

- Inspect the iBind™ card before use. Bends, creases, or prominent wrinkles in the card can result in poor immunodetection. See the user manual for additional guidance.
- Ensure the membrane is placed **protein-side down** and the wells are not positioned over the membrane when the lid of the iBind device is closed.
- Use the Blotting Roller to firmly roll the membrane on the iBind card. When rolling firmly, the iBind card will dip in the membrane area, causing the top and bottom of the card to slightly lift. See the user manual for additional guidance.
- If you mark your membrane with ink, mark the membrane near the low molecular weight region.
- Do not move the iBind™ device or open the lid until the incubation is complete (2.5 hours to overnight).

Prepare solutions and membranes

1. Prepare the necessary volume of 1X iBind™/iBind™ FD Solution. For example, if 30 mL of iBind™/iBind™ FD Solution is needed, prepare the solution according to the table below.

Component	Volume for chemiluminescent detection	Volume for fluorescent detection ¹
100X Additive	300 µL	75 µL
iBind™/iBind™ FD 5X Buffer	6 mL	6 mL
Distilled water	23.7 mL	23.9 mL

¹ If using the optional 1X iBind™ FD Solution, add 300 µL of 100X Additive and 1.5 mL of iBind™ FD 5X Buffer to 28.2 mL of distilled water.

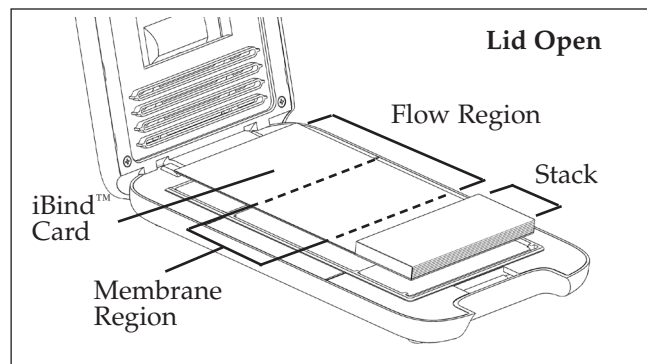
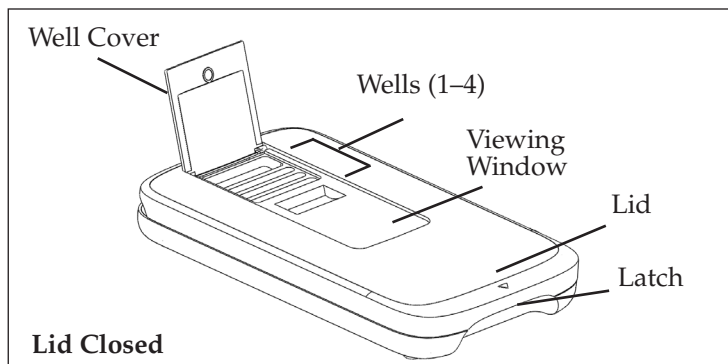
2. To block the membrane, immerse the blotted membrane (**protein-side up**) in 5 mL of 1X iBind™/iBind™ FD Solution. Incubate for 2–10 minutes with or without shaking.
3. Prepare the antibody solutions.

Prepare primary antibody solution	
1X iBind™/iBind™ FD Solution	2 mL
1° Antibody	Dilute the primary antibody to 2X the manufacturer's recommended dilution ¹ (for example, use a 1:500 dilution if a 1:1,000 dilution is recommended).
Prepare secondary antibody solution	
1X iBind™/iBind™ FD Solution	2 mL
iBind™ FD 10% SDS ²	10 µL
2° Antibody	Dilute the secondary antibody to 10X the manufacturer's recommended dilution ¹ (for example, use a 1:8,000 dilution if a 1:80,000 dilution is recommended).

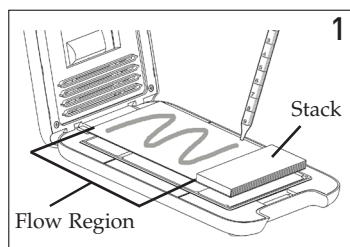
¹ Recommended starting dilutions. Antibody dilutions can be adjusted to achieve the desired signal.

² Used only with the iBind™ Fluorescent Detection (FD) Solution Kit (Cat. No. SLF1019).

Description of parts

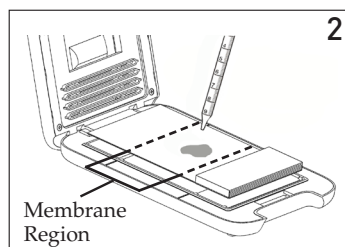


Western blot procedure

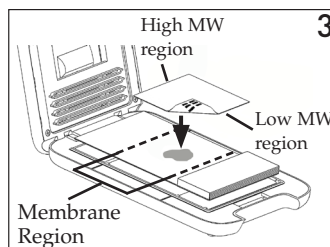


1. Place the iBind™ Card on the stage and pipette 5 mL of 1X iBind™/iBind™ FD Solution evenly across the Flow Region.

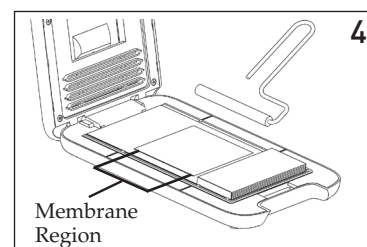
Note: Do not wet the stack.



2. Add 1 mL of 1X iBind™/iBind™ FD Solution so that it pools at the center of the membrane region

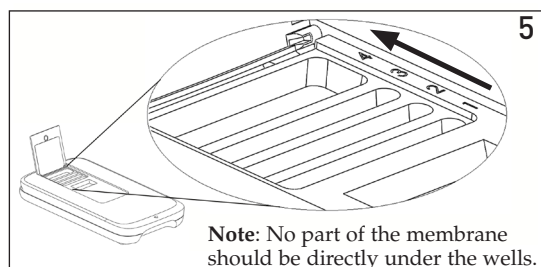


3. Place the membrane on top of the pooled solution with the **protein-side down** and the low molecular weight region closest to the stack.



4. Use the Blotting Roller to firmly roll membrane on the iBind™ Card to ensure good contact and remove any air bubbles.

Note: When firmly rolling, the iBind™ Card will slightly dip in the membrane area causing the top and bottom of the card to lift.



5. Close the lid and sequentially add solutions to the wells starting with well 1 (see Table 1 for solutions). Close the well cover.


Table 1. Solutions to add to wells in step 5.

<p>⚠ Add solutions in the following order:</p> <p>Well 1: 2 mL diluted 1° antibody</p> <p>Well 2: 2 mL iBind™/iBind™ FD Solution</p> <p>Well 3: 2 mL diluted 2° antibody</p> <p>Well 4: 6 mL iBind™/iBind™ FD Solution</p>
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6. Incubate for 2.5 hours to overnight. When incubation is complete, rinse the membrane in water and proceed to detection protocol.

Maintenance

Rinse the iBind™ Western Device under running water after each use and allow the device to dry before additional usage. To maximize device lifespan, store the iBind™ Western Device with the latch unlocked and the lid open.

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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