

iBind™ Western System

Catalog Number SLF1000

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Instructions for using the iBind™ Western Device in a western blot workflow with the iBind™ PLUS Solution Kit and iBind™ Fluorescent Detection (FD) Solution Kit are described below. Solution kits and iBind™ Cards are sold separately. For detailed instructions and guidance on optimizing results, refer to the user manual available from thermofisher.com/ibind.

General guidelines

- Use the iBind™ PLUS Solution Kit (Cat. No. SLF1030) if performing chemiluminescent or colorimetric detection.
- Use the iBind™ Fluorescent Detection (FD) Solution Kit (Cat. No. SLF1019) if performing fluorescent detection.
- 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 solutions according to the workflow being performed.

Chemiluminescent detection

The iBind™ PLUS Solution Kit contains two ready-to-use solutions that do not require preparation.

- Use iBind™ PLUS Solution to block membranes, wet iBind™ Cards, and in all wash steps.
- Use iBind™ PLUS Antibody Diluent to dilute primary and secondary antibodies.

Fluorescent detection

Prepare the required volume of 1X iBind™ FD Solution with the components in the iBind™ Fluorescent Detection (FD) Solution Kit. 30 mL of solution is required to process each membrane.

| Component | Volume |
|---------------------|---------|
| 100X Additive | 75 µL |
| iBind™ FD 5X Buffer | 6 mL |
| Distilled water | 23.9 mL |

2. Block the blotted membrane by immersing it (**protein-side up**) in 10 mL of iBind™ PLUS Solution (chemiluminescent detection), or 1X iBind™ FD Solution (fluorescent detection). Incubate for 2–10 minutes with or without shaking.
3. Prepare the antibody solutions using iBind™ PLUS Antibody Diluent (chemiluminescent detection), or 1X iBind™ FD Solution (fluorescent detection).

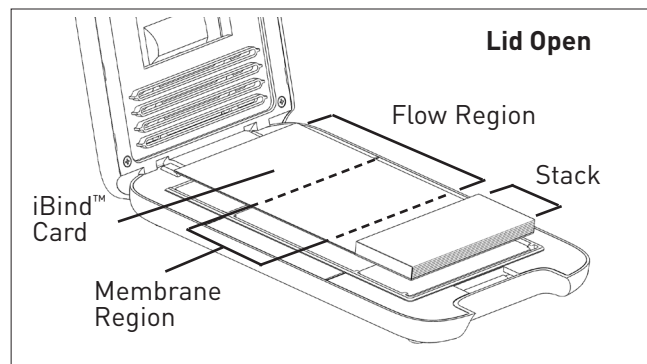
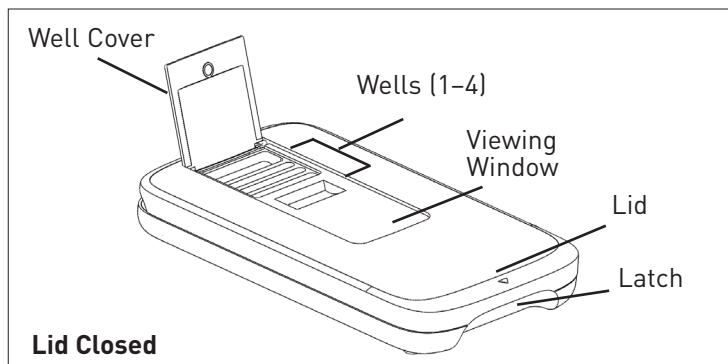
| Primary antibody solution | |
|---|---|
| iBind™ PLUS Antibody Diluent or 1X iBind™ FD Solution | 2 mL |
| Primary Antibody | <p>Chemiluminescent detection: Add the antibody at the manufacturer recommended dilution ^[1]. For example, use 2 µL (1:1,000 dilution) if a 1:1,000 dilution is recommended.</p> <p>Fluorescent detection: Add the antibody at 2X the manufacturer recommended dilution ^[1]. For example, use 1 µL (1:500 dilution) if a 1:1,000 dilution is recommended.</p> |

| Secondary antibody solution | |
|---|--|
| iBind™ PLUS Antibody Diluent or 1X iBind™ FD Solution | 2 mL |
| iBind™ FD 10% SDS ^[2] | 10 µL |
| Secondary Antibody | Add the antibody at 10X the manufacturer recommended dilution ^[1] . For example, use 2 µL (1:1,000 dilution) if a 1:10,000 dilution is recommended. |

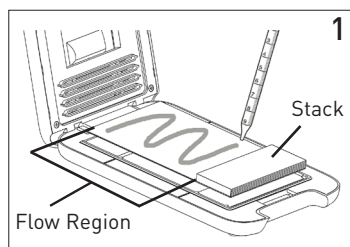
[1] Recommended starting dilutions. Antibody dilutions can be adjusted to achieve the desired signal.

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

Description of parts

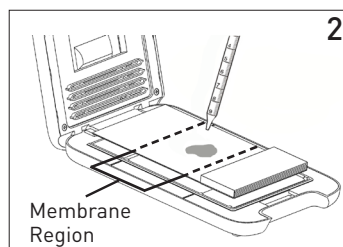


Western blot procedure

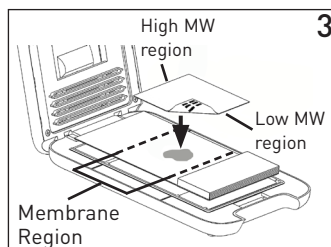


1. Place the iBind™ Card on the stage and pipette 5 mL of iBind™ PLUS/1X iBind™ FD Solution evenly across the Flow Region.

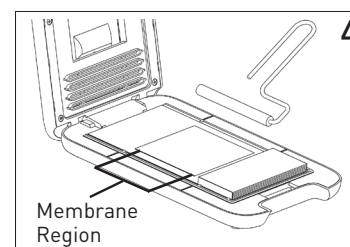
Note: Do not wet the stack.



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

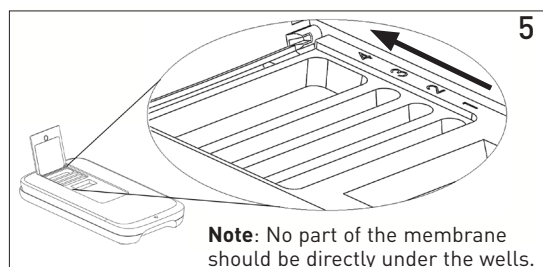


3. Place the membrane on the pooled solution with the **protein-side down** and the low molecular weight region closest to the stack.



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.



Note: No part of the membrane should be directly under the wells.

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

△ Add solutions in the following order:

Well 1: 2 mL diluted primary antibody

Well 2: 2 mL iBind™ PLUS/1X iBind™ FD Solution

Well 3: 2 mL diluted secondary antibody

Well 4: 6 mL iBind™ PLUS/1X iBind™ FD Solution

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

6. Incubate for 2.5 hours to overnight. Open the well cover to confirm incubation is complete (all reagents have drained from the wells). 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.

Multiple Life Technologies Corporation manufacturing sites are responsible for manufacturing the products associated with the workflow covered in this guide. For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Limited product warranty

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