iBind™ Flex Western System

Catalog Number SLF2000

Publication No. MAN0010926 **Rev.** D

Instructions for using the iBind™ Flex 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™ Flex 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™ Flex device is closed.
- Use the Blotting Roller to firmly roll the membrane on the iBind™ Flex card. When firmly rolling, the iBind™ Flex 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™ Flex device or open the lid until the incubation is complete (2.5 hours to overnight).
- Select a well insert based on the blot size being processed and place it into the iBind™ Flex device:
  - **Midi insert** – Single midi-sized membrane
  - **Mini insert** – 1 or 2 mini-sized membranes
  - **Multi-strip insert** – Up to 6 vertically cut membrane strips (**Not recommended for membranes cut into horizontal strips)**

Prepare solutions and membranes

1. Prepare the necessary volume of 1X iBind™ Flex/iBind™ Flex FD Solution. For example, if 50 mL of iBind™ Flex/iBind™ Flex FD Solution is needed, prepare the solution according to the table below.

Component	Volume for chemiluminescent detection	Volume for fluorescent detection[1]
100X Additive	500 µL	125 µL
iBind™ Flex/iBind™ Flex FD 5X Buffer	10 mL	10 mL
Distilled water	39.5 mL	39.9 mL

[1] If using the optional 1X iBind™ Flex FD Solution, add 500 µL of 100X Additive and 2.5 mL of iBind™ Flex FD 5X Buffer to 47 mL of distilled water.

2. To block the membrane, immerse the blotted membrane (**protein-side up**) in 1X iBind™ Flex/iBind™ Flex FD Solution. Use 10–20 mL for midi sized membranes or vertically cut strips, or 5–10 mL for each mini-sized membrane based on the size of the incubation tray. Ensure blot is fully submerged in solution. Incubate for 2–10 minutes, with or without shaking.
3. Prepare the antibody solutions.

Prepare primary antibody solution			
Component	Midi blot	Mini blot	Vertical strip
1X iBind™ Flex/iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
1° Antibody	Dilute the primary antibody to 2X the manufacturer's recommended dilution[1] (for example, 1:500 if 1:1,000 dilution is recommended.		
Prepare secondary antibody solution			
Component	Midi blot	Mini blot	Vertical strip
1X iBind™ Flex/iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
iBind™ Flex FD 10% SDS[2]	20 µL	10 µL	3.5 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 iBind™ Flex Fluorescent Detection (FD) Solution Kit (Cat. No. SLF2019).

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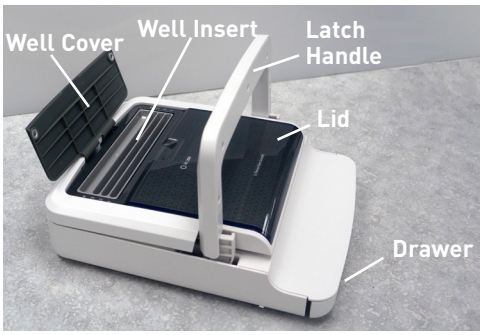
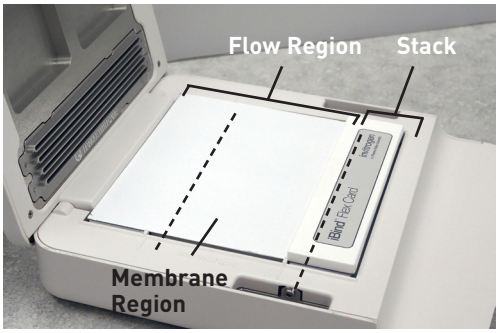
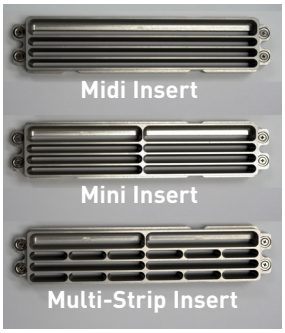
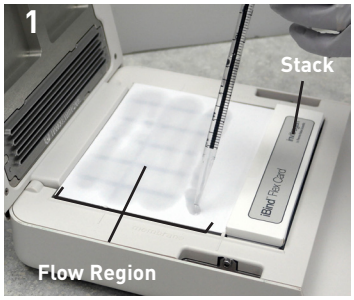
Description of parts



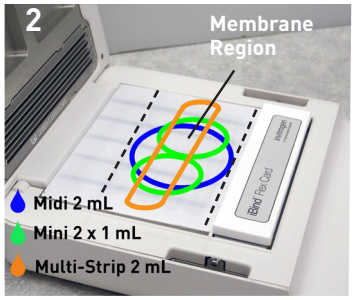




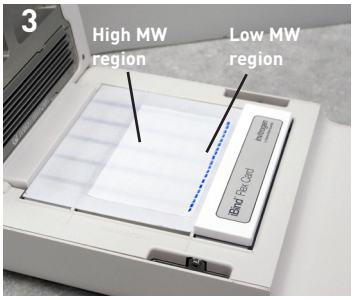
Western blot procedure



1. Place the iBind™ Flex Card on the stage and pipette 10 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution across the Flow Region. Lines appear to help align membranes with wells. **Note: Do not wet the Stack.**



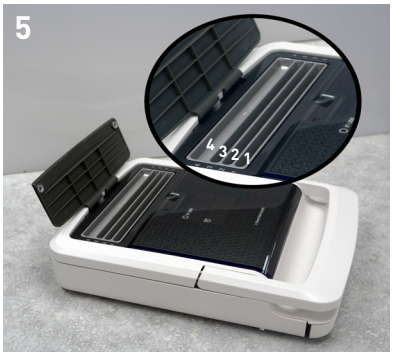
2. Add 1X iBind™ Flex/iBind™ Flex FD Solution based on the size of the membrane so that it pools in the indicated regions on the iBind™ Flex Card.



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™ Flex Card to ensure good contact and remove any air bubbles.

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



5. Close the lid and add solutions sequentially 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.

Add solutions in the following order:	Volume/Well		
	Midi Blot	Mini Blot	Vertical Strip
Row 1: Diluted 1° antibody	4 mL	2 mL	0.7 mL
Row 2: iBind™ Flex/iBind™ Flex FD Solution	4 mL	2 mL	2 mL
Row 3: Diluted 2° antibody	4 mL	2 mL	0.7 mL
Row 4: iBind™ Flex/iBind™ Flex FD Solution	12 mL	6 mL	6 mL

6. Incubate for 2.5 hours to overnight. When incubation is complete, rinse the membrane in water and proceed to detection protocol.

Note: Ensure no membrane part is directly under the wells.

Maintenance

Handle well inserts with care. Rinse the iBind™ Flex well inserts under running water after each use and allow to dry before additional usage. Store inserts in the drawer of the iBind™ Flex Western Device

To maximize device lifespan, store the iBind™ Flex 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.

For support visit thermofisher.com/support or email techsupport@lifetech.com thermofisher.com

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