iBind™ Flex Western System

Catalog Number SLF2000

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Instructions for using the iBind™ Flex Western Device in a western blot workflow with the iBind™ Flex PLUS Solution Kit and iBind™ Flex Fluorescent Detection (FD) Solution Kit are described below. Solution kits and iBind™ Flex 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™ Flex PLUS Solution Kit (Cat. No. SLF2030) if performing chemiluminescent or colorimetric detection.
- Use the iBind™ Fluorescent Detection (FD) Solution Kit (Cat. No. SLF1019) if performing fluorescent detection.
- 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 (see **Description of parts**).

Prepare solutions and membranes

1. Prepare solutions according to the workflow being performed.

Chemiluminescent detection

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

- Use iBind™ Flex PLUS Solution to block membranes, wet iBind™ Flex Cards, and in all wash steps.
- Use iBind™ Flex 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. 50 mL of solution is required for each processing run.

Component	Volume
100X Additive	125 µL
iBind™ Flex FD 5X Buffer	10 mL
Distilled water	39.9 mL

2. Block the blotted membrane by immersing it (**protein-side up**) in iBind™ Flex PLUS Solution (chemiluminescent detection), or 1X iBind™ Flex FD Solution (fluorescent detection). Use 20 mL for midi sized membranes or vertically cut strips, or 10 mL for a mini-sized membrane. Ensure blot is fully submerged in solution. Incubate for 2–10 minutes, with or without shaking.

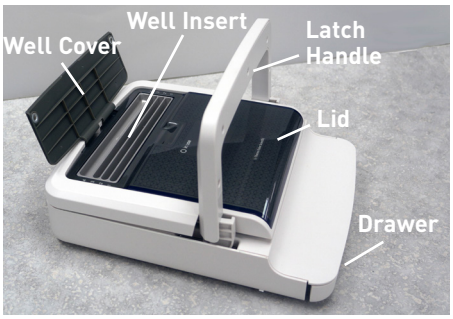
3. Prepare the antibody solutions.

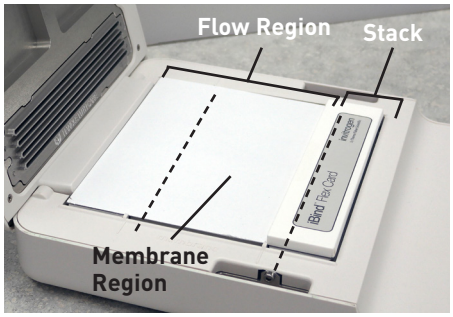
Components for Primary Antibody Solution	Midi blot	Mini blot	Vertical strip
iBind™ Flex PLUS Antibody Diluent/1X iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
Primary Antibody	**Chemiluminescent detection:** Add antibody at the manufacturer recommended dilution [1]. For example, a mini blot needs 2 µL (1:1,000 dilution) if a 1:1,000 dilution is recommended. **Fluorescent detection:** Add antibody at 2X the manufacturer recommended dilution [1]. For example, a mini blot needs 1 µL (1:500 dilution) if a 1:1,000 dilution is recommended.		

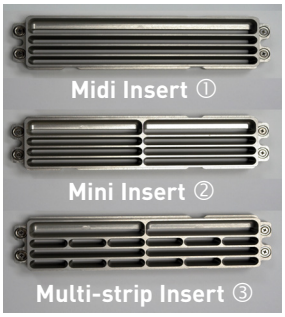
Components for Secondary Antibody Solution	Midi blot	Mini blot	Vertical strip
iBind™ Flex PLUS Antibody Diluent/1X iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
iBind™ Flex FD 10% SDS[2]	20 µL	10 µL	3.5 µL
Secondary Antibody	Add the antibody at 10X the manufacturer recommended dilution [1]. For example, a mini blot needs 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





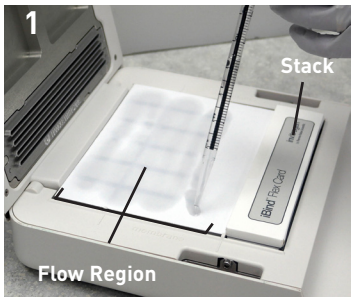


① Single midi-sized membrane

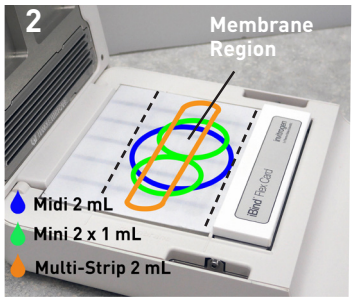
② 1 or 2 mini-sized membranes

③ Up to 6 vertically cut membrane strips **(Not recommended for membranes cut into horizontal strips)**

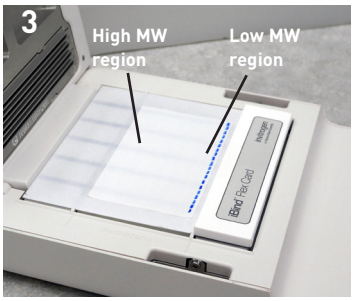
Western blot procedure



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



2. Add iBind™ Flex PLUS/1X 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.

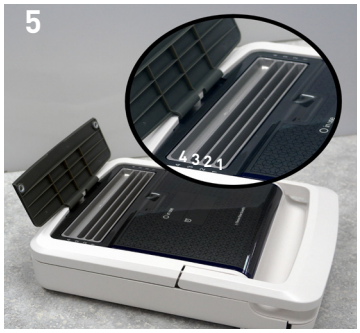


Table 1. Solutions to add to wells in step 5.

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

5. Close the lid and add solutions to the wells in sequence starting with well 1 (see Table 1 for solutions). Close the well cover.
   Note: Ensure no membrane part is directly under the wells.

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

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.

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.

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

25 February 2026