

iBind™ Flex Western System

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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 manual available from thermofisher.com/ibind.

General Guidelines

- Store membranes in iBind™ Flex/iBind™ Flex FD Solution, in distilled water, or dry.
- If you mark your membrane with ink, mark the membrane near the low molecular weight region.
- **Important!** Make sure that the wells are not positioned over the membrane when the lid of the iBind™ Flex device is closed.
- 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** – membranes cut into vertical strips (**Not recommended** for membranes cut into **horizontal strips**).
- Perform the western detection protocol according to the following steps:
 - Prepare solutions (fluorescent detection protocol or HRP or AP detection protocol).
 - Perform western blot procedure and detection.

Prepare solutions

HRP or AP detection

1. Prepare 1X iBind™ Flex Solution:

| Component | Volume |
|-----------------------|---------|
| 100X Additive | 500 µL |
| iBind™ Flex 5X Buffer | 10 mL |
| Distilled Water | 39.5 mL |

2. Immerse blotted membrane in 10 mL 1X iBind™ Flex Solution.

3. Prepare primary antibody solutions:

| Component | Midi Blot | Mini Blot | Vertical Strip |
|-------------------------|---|-----------|----------------|
| 1X iBind™ Flex Solution | 4 mL | 2 mL | 0.7 mL |
| 1° Antibody | Dilute antibody to manufacturer's recommended dilution. | | |

4. Prepare secondary antibody solutions:

| Component | Midi Blot | Mini Blot | Vertical Strip |
|-------------------------|--|-----------|----------------|
| 1X iBind™ Flex Solution | 4 mL | 2 mL | 0.7 mL |
| 2° Antibody | Use final antibody concentration at 5x the manufacturer's recommended dilution. (e.g. 1:1000 dilution if 1:5000 dilution recommended). | | |

Fluorescent detection

1. Prepare 1X iBind™ Flex FD Solution*:

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

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

2. Immerse blotted membrane in 10 mL 1X iBind™ Flex FD Solution.

3. Prepare primary antibody solutions:

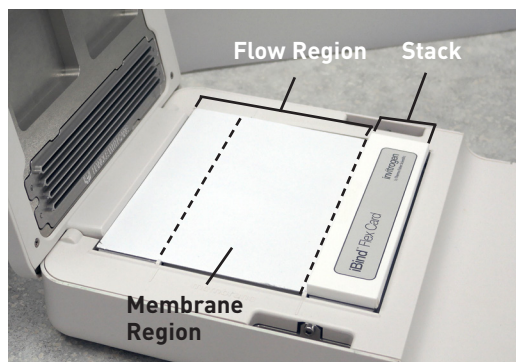
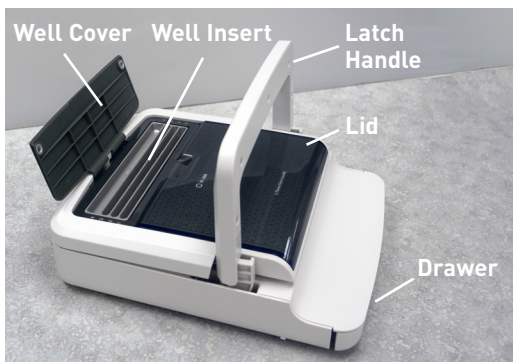
| Component | Midi Blot | Mini Blot | Vertical Strip |
|----------------------------|---|-----------|----------------|
| 1X iBind™ Flex FD Solution | 4 mL | 2 mL | 0.7 mL |
| 1° Antibody | Dilute antibody to manufacturer's recommended dilution. | | |

4. Prepare secondary antibody solutions:

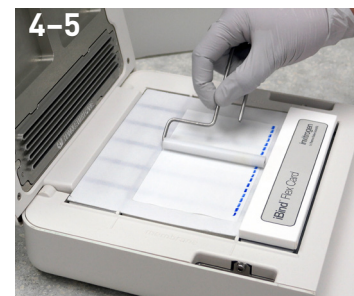
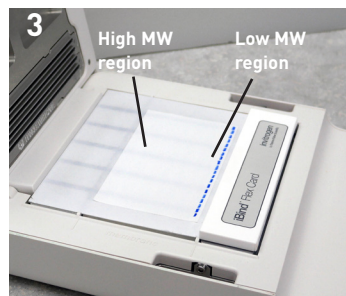
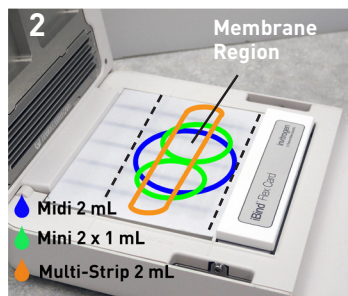
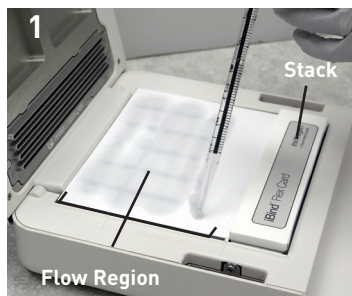
| Component | Midi Blot | Mini Blot | Vertical Strip |
|--|--|---|--|
| 1X iBind™ Flex FD Solution | 4 mL | 2 mL | 0.7 mL |
| iBind™ Flex FD 10% SDS | 20 µL | 10 µL | 3.5 µL |
| <ul style="list-style-type: none"> ▪ Alexa Fluor® 680 OR ▪ IRDye® 680LT | <ul style="list-style-type: none"> ▪ 2 µL (1:2000 dilution) ▪ 1 µL (1:4000 dilution) | <ul style="list-style-type: none"> ▪ 1 µL (1:2000 dilution) ▪ 0.5 µL (1:4000 dilution) | <ul style="list-style-type: none"> ▪ 0.35 µL (1:2000 dilution) ▪ 0.18 µL (1:4000 dilution) |
| <ul style="list-style-type: none"> ▪ Alexa Fluor® 790 OR ▪ IRDye® 800CW | <ul style="list-style-type: none"> ▪ 2 µL (1:2000 dilution) ▪ 1.3 µL (1:3000 dilution) | <ul style="list-style-type: none"> ▪ 1 µL (1:2000 dilution) ▪ 0.67 µL (1:3000 dilution) | <ul style="list-style-type: none"> ▪ 0.35 µL (1:2000 dilution) ▪ 0.23 µL (1:3000 dilution) |

For Research Use Only. Not for use in diagnostic procedures.

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 and **firmly roll** to remove air bubbles and ensure good contact between the membrane and iBind™ Flex Card.

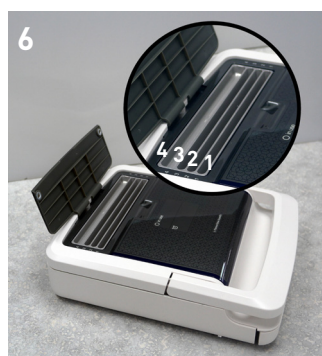
5. Close the iBind™ Flex device lid and lower latch handle to lock the lid.

6. Add solutions sequentially to each well starting with Row 1 (see Table 1).

7. Close the well cover and record the time for the start of incubation.

8. Incubate 2.5 hours to overnight.

9. Rinse the membrane in water and proceed to immunodetection protocol.



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

Table 1

| 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 |

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.

Store the iBind™ Flex Western Device with the latch unlocked, and the lid not fully closed.

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