

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	Use final antibody concentration equal to 5x the manufacturer's recommended dilution (e.g. 1:200 if 1:1000 dilution recommended).		

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.

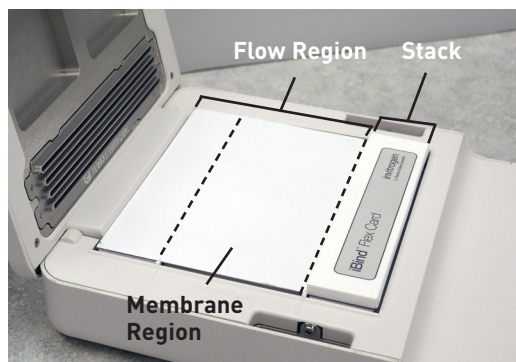
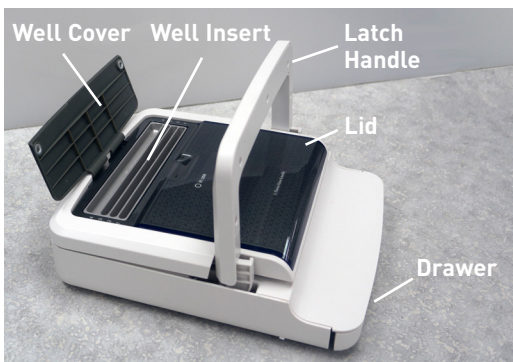
2. Immerse blotted membrane in 10 mL 1X iBind™ Flex FD Solution.
3. Prepare primary antibody solutions:

Component	Midi Blot	Mini Blot	Vertical Strip
1X iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
1° Antibody	Use final antibody concentration equal to 5x the manufacturer's recommended dilution (e.g. 1:200 if 1:1000 dilution recommended).		

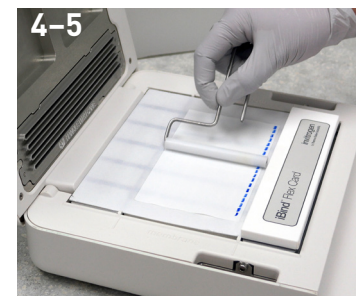
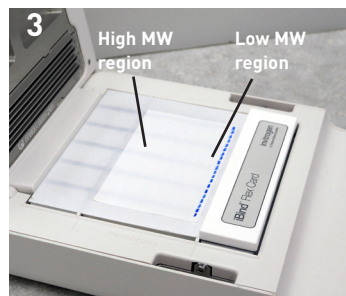
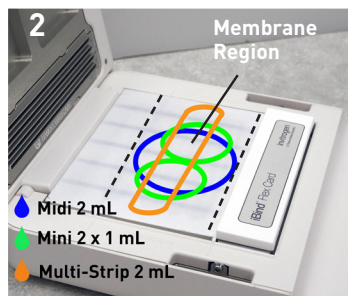
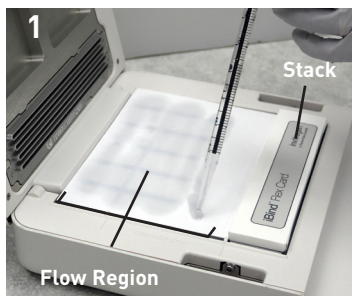
4. Prepare secondary antibody solutions:

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)

Description of parts



Western blot procedure



- 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.

- 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.

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

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

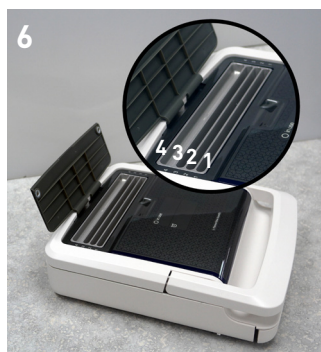
- Close the iBind™ Flex device lid and lower latch handle to lock the lid.

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

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

- Incubate 2.5 h to overnight.

- 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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