

iBind™ Western System

Catalog Number SLF1000

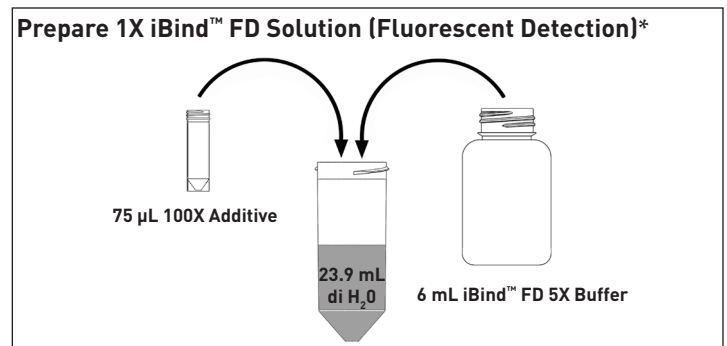
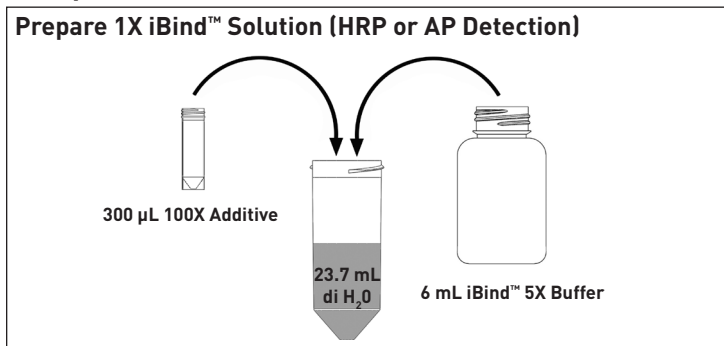
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Instructions for using the iBind™ Western Device in a western blot workflow are described below. For detailed instructions and guidance on optimizing results, refer to the manual available from thermofisher.com/ibind.

General Guidelines

- Store membranes in iBind™/iBind™ 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™ device is closed.
- Do not move the iBind™ device or open the lid until the incubation is complete (2.5 hours to overnight).
- Perform the western detection protocol according to the following steps:
 - Prepare 1X iBind™ Solution (HRP or AP detection) or 1X iBind™ FD Solution (fluorescent detection).
 - Prepare membrane (membranes should only be blocked with iBind™/iBind™ FD Solution).
 - Prepare diluted antibody solutions.
 - Perform western blot procedure and detection.

Prepare solutions



* For the Optional 1X iBind™ FD Solution, add 300 µL 100X Additive, and 1.5 mL iBind™ FD 5X Buffer to 28.2 mL distilled water.

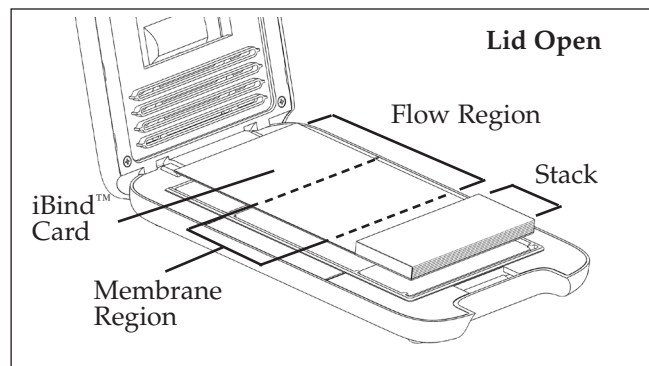
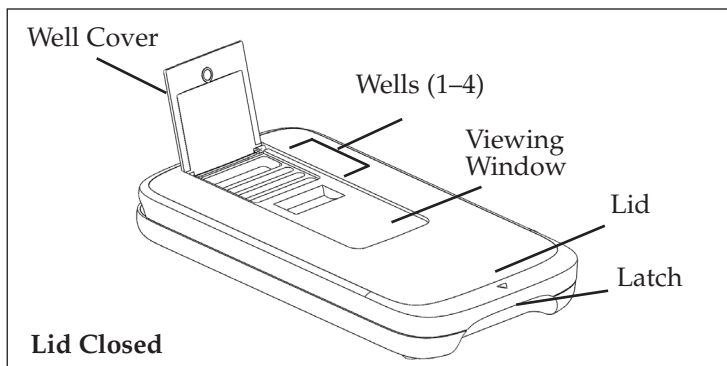
Table 1.1 Prepare primary antibody solutions

Component	HRP detection
1X iBind™ Sol'n	2 mL
1° Antibody	Final antibody concentration equal to the manufacturer's recommended dilution.
Component	Novex® AP Detection Kit only
1X iBind™ Sol'n	2 mL
1° Antibody	Final antibody concentration equal to the manufacturer's recommended dilution.
Component	Fluorescent detection
1X iBind™ FD Sol'n	2 mL
1° Antibody	Final antibody concentration equal to the manufacturer's recommended dilution.

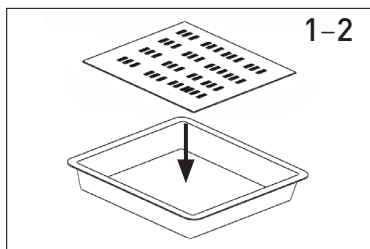
Table 1.2 Prepare secondary antibody solutions

Component	HRP detection
1X iBind™ Sol'n	2 mL
2° Antibody	Final antibody concentration at 5X the manufacturer's recommended dilution.
Component	Novex™ AP Detection Kit only
1X iBind™ Sol'n	2 mL
▪ Anti-Mouse 2° Antibody OR ▪ Anti-Rabbit 2° Antibody	▪ 2 µL (1:1000 dilution) ▪ 1 µL (1:2000 dilution)
Component	Fluorescent detection
1X iBind™ FD Sol'n	2 mL
iBind™ FD 10% SDS	10 µL
▪ Alexa Fluor™ 680 OR ▪ IRDye™ 680LT	▪ 1 µL (1:2000 dilution) ▪ 0.5 µL (1:4000 dilution)
▪ Alexa Fluor™ 790 OR ▪ IRDye™ 800CW	▪ 1 µL (1:2000 dilution) ▪ 0.67 µL (1:3000 dilution)

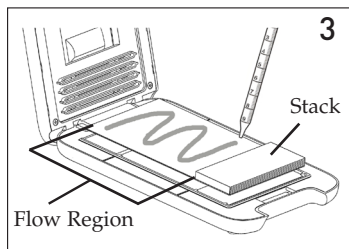
Description of parts



Western blot procedure

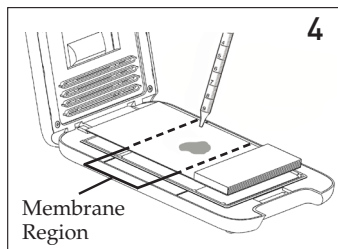


1. Immerse blotted membrane in 5 mL 1X iBind™ /iBind™ FD Solution.
2. Dilute antibodies right after wetting the membrane (refer to Table 1).

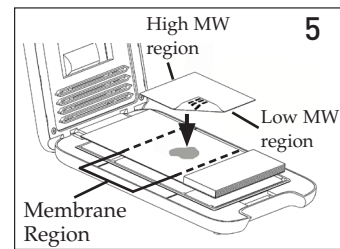


3. Place the iBind™ Card on the stage and pipette 5 mL of 1X iBind™ /iBind™ FD Solution evenly across the Flow Region.

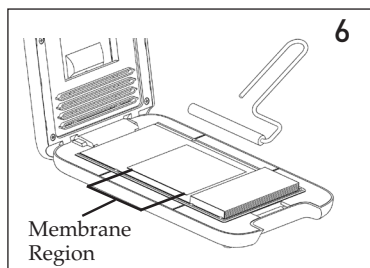
Note: Do not wet the Stack.



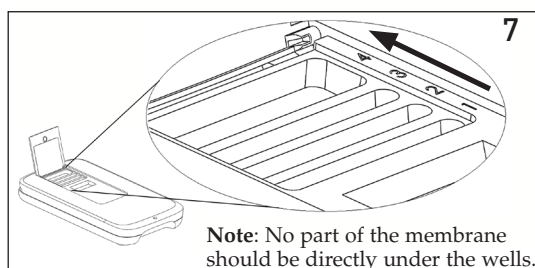
4. Add 1 mL of 1X iBind™ / iBind™ FD Solution so that it pools at the center of the membrane region on the iBind™ Card.



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



6. Use the Blotting Roller to remove any air bubbles.



7. Close the lid and add solutions sequentially to the iBind™ Wells starting with Well 1 (see Table 2).
8. Place the window cover over the viewing window, and close the well cover. Incubate 2.5 h to overnight.
9. Rinse the membrane in water and proceed to immunodetection protocol.

Table 2

▲	Add solutions in the following order:
Well 1:	2 mL diluted 1° antibody
Well 2:	2 mL iBind™/iBind™ FD Solution
Well 3:	2 mL diluted 2° antibody
Well 4:	6 mL iBind™/iBind™ FD Solution

Maintenance

Rinse the iBind™ Western Device under running water after each use and allow the device to dry before additional usage. Store the iBind™ Western Device with the latch unlocked, and the lid open.

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