

iBind™ Western System

Catalog Number SLF1000

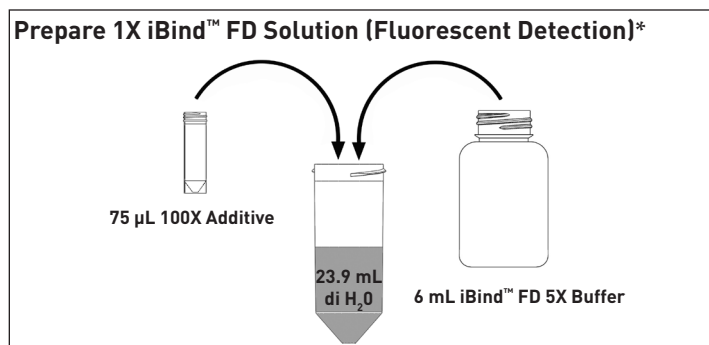
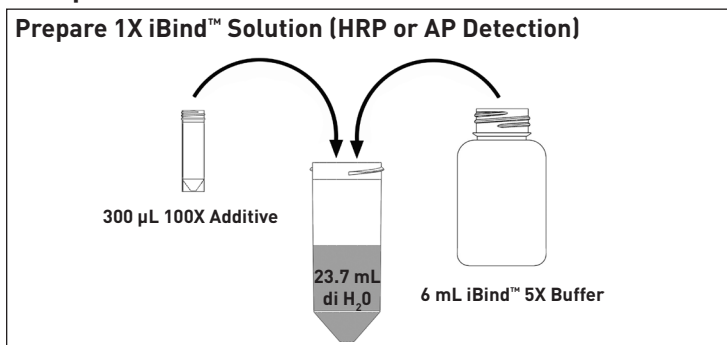
Publication Part No. 25-1075 Publication No. MAN0008932 Rev. E.0

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

- We recommend moving directly from transfer to the iBind™ procedure for blocking and antibody binding. If storage before processing is required, then 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 diluted antibody solutions.
 - Perform iBind™ procedure followed by 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.

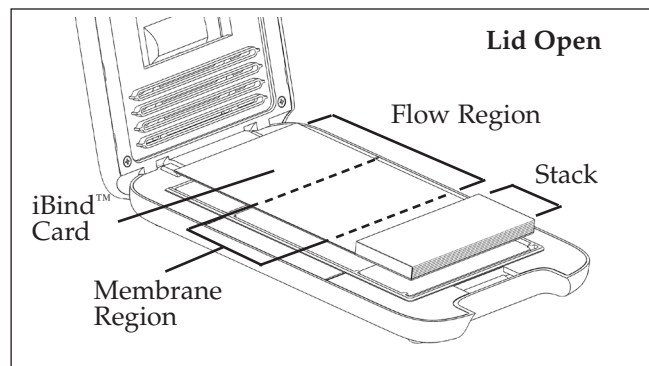
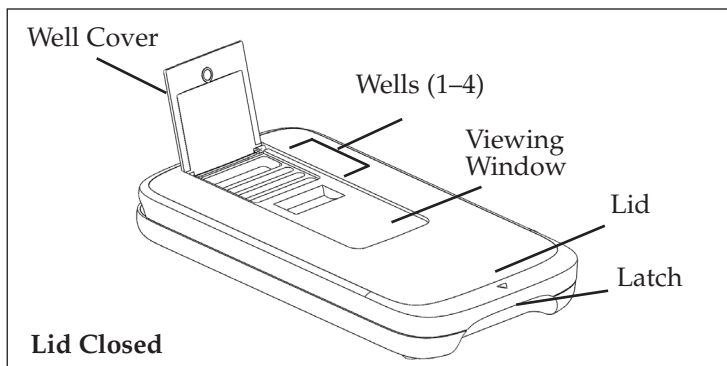
Table 1.1 Prepare primary antibody solutions

Component	HRP detection
1X iBind™ Sol'n	2 mL
1° Antibody	Use final antibody concentration equal to 1x-5x the manufacturer's recommended dilution (e.g. 1:200 if 1:1000 dilution recommended).
Component	Fluorescent detection
1X iBind™ FD Sol'n	2 mL
1° Antibody	Use final antibody concentration equal to 1x-5x the manufacturer's recommended dilution (e.g. 1:200 if 1:1000 dilution recommended). A starting concentration of 0.5x is recommended. Optimize to lower primary concentrations based on initial results.

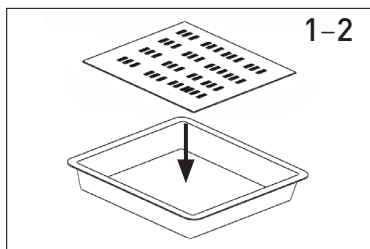
Table 1.2 Prepare secondary antibody solutions

Component	HRP detection
1X iBind™ Sol'n	2 mL
2° Antibody	Use final secondary concentration equal to >5x the manufacturer's recommended dilution for the respective detection substrate used. Typical dilution of chemiluminescent label secondaries for iBind™ is 1:500-1:5000. Start at higher concentration and optimize to lower dilutions based on results.
Component	Fluorescent detection
1X iBind™ FD Sol'n	2 mL
iBind™ FD 10% SDS	10 µL
Alexa Fluor™- OR IRDye™-Labeled 2° Antibody	<ul style="list-style-type: none"> ▪ Use final secondary concentration equal to 5x the manufacturer's recommended dilution. Typical dilution for Fluorescent-conjugated secondaries for iBind™ is 1:1000-1:4000.

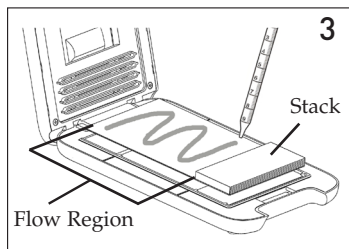
Description of parts



Western blot procedure

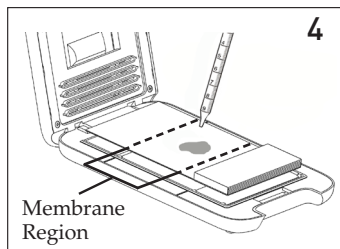


1. Immerse blotted membrane in 5 mL 1X iBind™ /iBind™ FD Solution.
2. Dilute antibodies right after wetting the membrane (refer to Table 1).

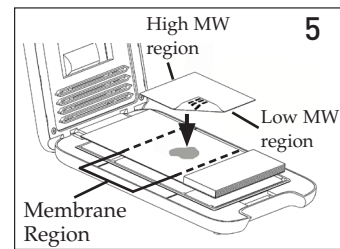


3. Place the iBind™ Card on the stage and pipette 5 mL of 1X iBind™ /iBind™ FD Solution evenly across the Flow Region.

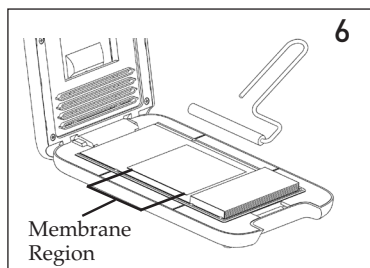
Note: Do not wet the Stack.



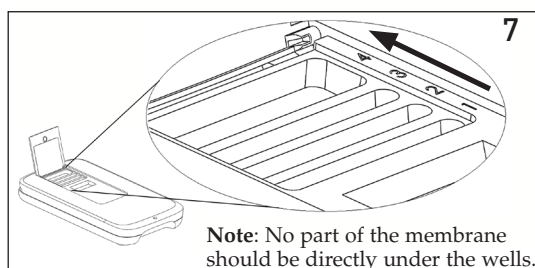
4. Add 1 mL of 1X iBind™ / iBind™ FD Solution so that it pools at the center of the membrane region on the iBind™ Card.



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 and slowly and firmly roll to remove air bubbles and ensure good contact between the membrane and iBind™ Flex Card. Use care to avoid roller edge cutting or damaging iBind™ Card fibers.
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 the detection protocol.



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

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.

DISCLAIMER TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

©2020 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

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

18 March 2020

ThermoFisher
SCIENTIFIC