invitrogen

iBind™ Flex Western System

USER GUIDE

For western detection of proteins on PVDF or nitrocellulose membranes

Catalog Number SLF2000

Publication Number MAN0010927

Revision C

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

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0010927 C (English)

Revision	Date	Description
C	10 February 2025	The guide content was edited to clarify procedures and usage for more optimal customer use.
B.0	20 August 2015	The guide content was edited with post-launch revisions.
A.0	5 May 2015	New document for iBind™ Flex Western System.

The information in this guide is subject to change without notice.

Contents

iBind™ Flex Western System

For western detection of proteins on PVDF or nitrocellulose membranes

Product information

Overview

The iBind™ Flex Western System is a benchtop device utilizing sequential lateral flow (SLF) to perform hands-free blocking, antibody binding, and washes for western detection workflows.

The iBind™ Flex Western System uses no external power source, and relies on mechanical pressure from the iBind™ Flex Western System on a iBind™ Flex Card to generate the sequential flow of immunodetection reagents for performing the blocking, antibody binding, and wash steps involved in western detection workflows (see Figure 1).

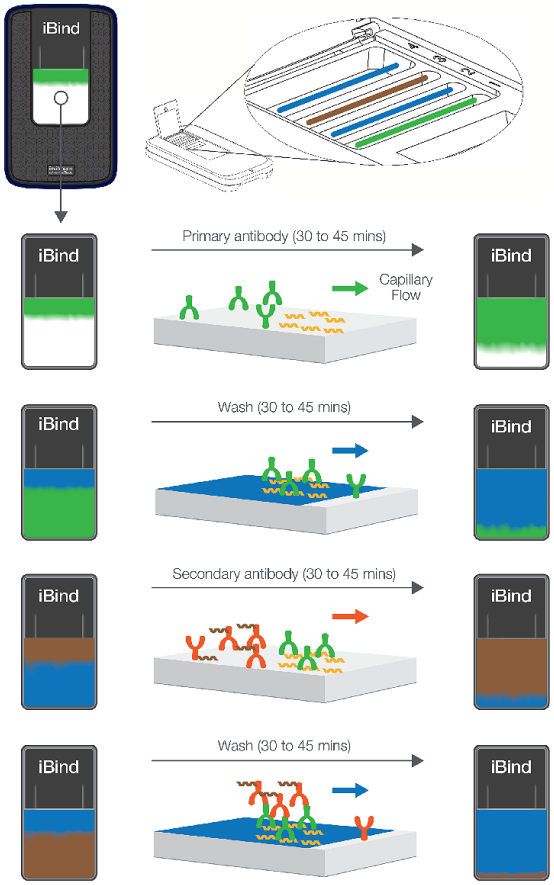


Figure 1 Sequential lateral flow technology employed by the iBind™ Flex Western System.

Figure 2 iBind™ Flex Western System

System components

The iBind™ Flex Western System consists of the following components:

- iBind™ Flex Western Device
- iBind™ Flex Midi Insert
- iBind™ Flex Mini Insert
- iBind™ Flex Multi-Strip Insert
- iBind™ Flex Cards
- iBind™ Flex Solution Kit
- iBind™ Flex Fluorescent Detection (FD) Solution Kit

Contents

The components included with the iBind™ Flex Western Device (Cat. No. SLF2000) are listed below.

Components	Quantity
iBind™ Flex Western Device	1 unit
iBind™ Blotting Roller	1 roller
iBind™ Flex Midi Insert	1 unit
iBind™ Flex Mini Insert	1 unit
iBind™ Flex Multi-Strip Insert	1 unit

Required materials not supplied with the device

The following components are used with the iBind™ Flex Western System, but not included with the device.

iBind™ Flex Cards

The iBind™ Flex Card is a unique matrix optimized for homogenous flow of immunodetection reagents along its length (see “iBind™ Flex Card” on page 14 for details).

The iBind™ Flex Cards are single use and sold separately (see “Related products” on page 50 for ordering details).

The components included with the iBind™ Flex Cards (Cat. No. SLF2010) are listed below.

Product	Quantity	Storage
iBind™ Flex Card	10 cards	Room temperature

iBind™ Flex Fluorescent Detection (FD) Solution Kit

The iBind™ Flex FD Solution Kit is used for preparing blocking, dilution, and washing buffers for the iBind™ Flex western detection protocol in conjunction with fluorophore-conjugated secondary antibodies (for example, Alexa Fluor™ Plus Secondary Antibodies). The iBind™ Flex Fluorescent Detection (FD) Solution Kit is sold separately (see “Related products” on page 50 for ordering details).

The components included with the iBind™ Flex Fluorescent Detection (FD) Solution Kit (Cat. No. SLF2019) are listed below and sufficient for 10 midi blots or 20 mini blots.[1]

Component	Quantity	Storage
iBind™ Flex FD 5X Buffer	100 mL	4°C
iBind™ Flex 100X Additive	3 x 1.7 mL	4°C
iBind™ Flex FD 10% SDS	200 µL	Room temperature

[1] The volume of solution used for blocking (10 mL to block midi blots/vertically cut strips or 5 mL to block mini blots) can be adjusted based on the size of the incubation tray being used. For more information, refer to the detection procedures beginning with “Fluorescent detection procedure” on page 23.

iBind™ Flex Solution Kit

The iBind™ Flex Solution Kit is used for preparing blocking, dilution, and washing buffers for the iBind™ Flex western detection protocol using chemiluminescent or colorimetric detection. The iBind™ Flex Solution Kit is sold separately (see “Related products” on page 50 for ordering details).

The components included with the iBind™ Flex Solution Kit (Cat. No. SLF2020) are listed below and sufficient for 10 midi blots or 20 mini blots.[2]

Component	Quantity	Storage
iBind™ Flex 5X Buffer	100 mL	4°C
iBind™ Flex 100X Additive	3 x 1.7 mL	4°C

Description of parts

iBind™ Flex Western Device

The iBind™ Flex Western Device is an automated device that uses sequential lateral flow (SLF) to automatically perform blocking, washing, and antibody incubation steps in a western detection workflow.

SLF allows the timely release and flow of solutions and antibodies to the membrane without need of an external power source. Each solution is released from iBind™ Flex wells to an iBind™ Flex Card via SLF. The glass fiber matrix of the card allows for homogenous and consistent flow of the solutions to the membrane, increasing the antigen-antibody interaction.

The iBind™ Flex well inserts have 4 rows of wells for loading blocking solution, antibodies, and wash solutions. There are 3 different well inserts (for processing one midi blot, up to 2 mini blots, or up to 6 vertically cut strip blots).

Lid and Drawer Closed

[2] The volume of solution used for blocking (10 mL to block midi blots/vertically cut strips or 5 mL to block mini blots) can be adjusted based on the size of the incubation tray being used. For more information, refer to the detection procedures beginning with “Chemiluminescent detection procedure” on page 20.

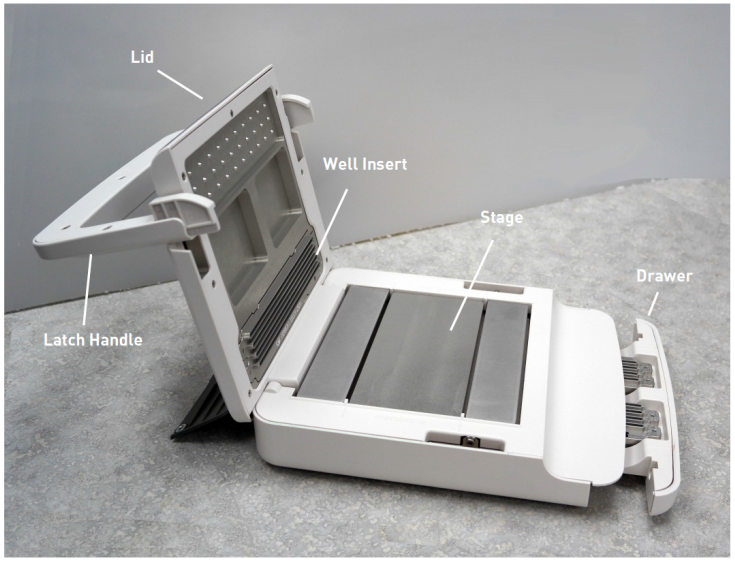
iBind™ Flex Western Device lid

The lid of the iBind™ Flex Western Device is designed to be marked with standard lab markers. A section is provided to mark the device as being “in use”, and record the time at which an incubation is started.

The iBind™ Flex Western Device consists of a metallic stage made up of three sections. The front and rear sections of the stage are spring plates designed to apply specific amounts of pressure on an iBind™ Flex Card placed on the stage when the lid of the device is locked.

The pressure on the iBind™ Flex Card results in the sequential flow of immunodetection reagents from the wells in which they are loaded. The flow rate is highly reproducible because the amount of pressure and the viscosity of the fluids remain constant.

Lid and Drawer Open



Drawer for storing iBind™ Flex well inserts

The iBind™ Flex well inserts are stored in a drawer at the front of the device.

Press on the front of the drawer to release the latch and slide the drawer open.

iBind™ Flex inserts

The iBind™ Flex Western Device has three interchangable inserts with different well configurations designed for processing different sizes of membranes.

Each insert has four rows which are filled with the following solutions:

Row	Solution
4	1X iBind™ Flex/ iBind™ Flex FD Solution
3	Diluted secondary antibody
2	1X iBind™ Flex/ iBind™ Flex FD Solution
1	Diluted primary antibody

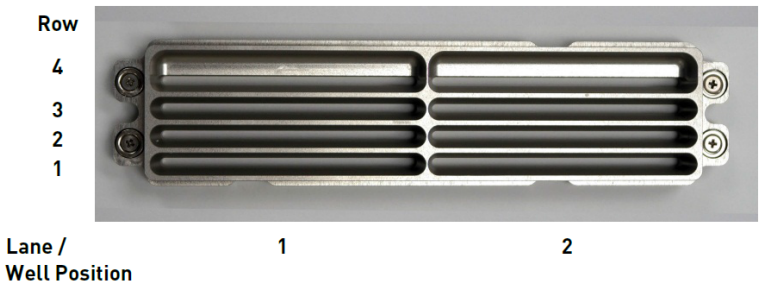
iBind™ Flex Midi Insert

The iBind™ Flex Midi Insert is designed for performing western detection protocols on midi-sized membranes.

iBind™ Flex Mini Insert

The iBind™ Flex Mini Insert is designed for performing western detection protocols on up to 2 mini-sized membranes.

Each row of the Mini Insert consists of 2 wells.

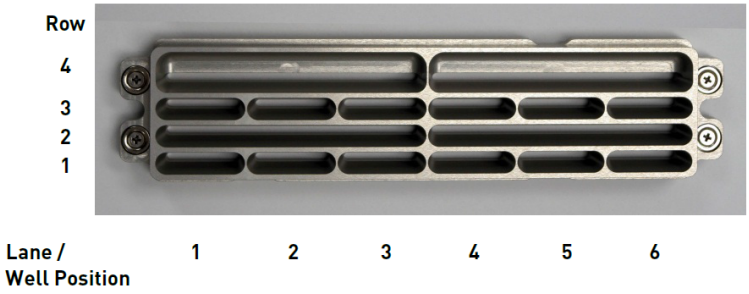


iBind™ Flex Multi-Strip Insert

The iBind™ Flex Multi-Strip Insert is designed for performing western detection protocols on up to 6 vertically cut membrane strips.

Rows 2 and 4 of the Multi-Strip Insert consist of 2 wells each, while rows 1 and 3 consist of 6 wells each.

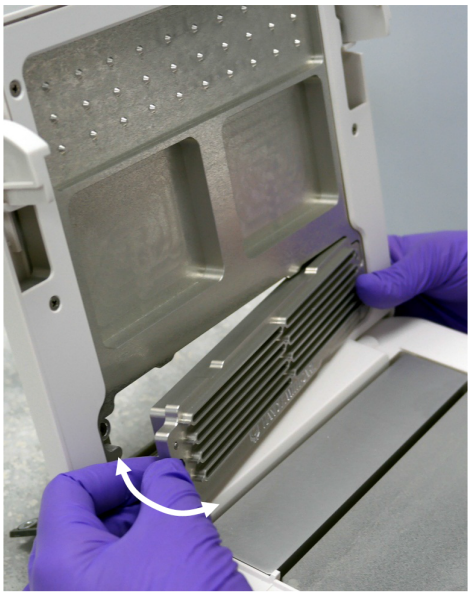
Refer to “Guidelines for vertically cut membrane strips” on page 19 for additional details on using the iBind™ Flex Multi-Strip Insert.



Remove/install an iBind™ Flex well insert

The iBind™ Flex well inserts are designed so that they only fit into the lid of the iBind™ Flex device in the correct orientation.

To remove an insert:	To install an insert:
1. Open the lid and well cover of the iBind™ Flex device. **2.** Push the insert from the back of the lid and slide it out of the slot.	**1.** Open the lid and well cover of the iBind™ Flex device. **2.** Slide the insert into the slot in the lid from the underside of the lid.



Blotting roller

The Blotting Roller is a plastic roller attached to a stainless steel handle (8.6 cm wide) and is used to remove air bubbles and ensure good contact between the membrane and the iBind™ Flex Card. **Firm rolling is required to ensure optimal results.**

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.

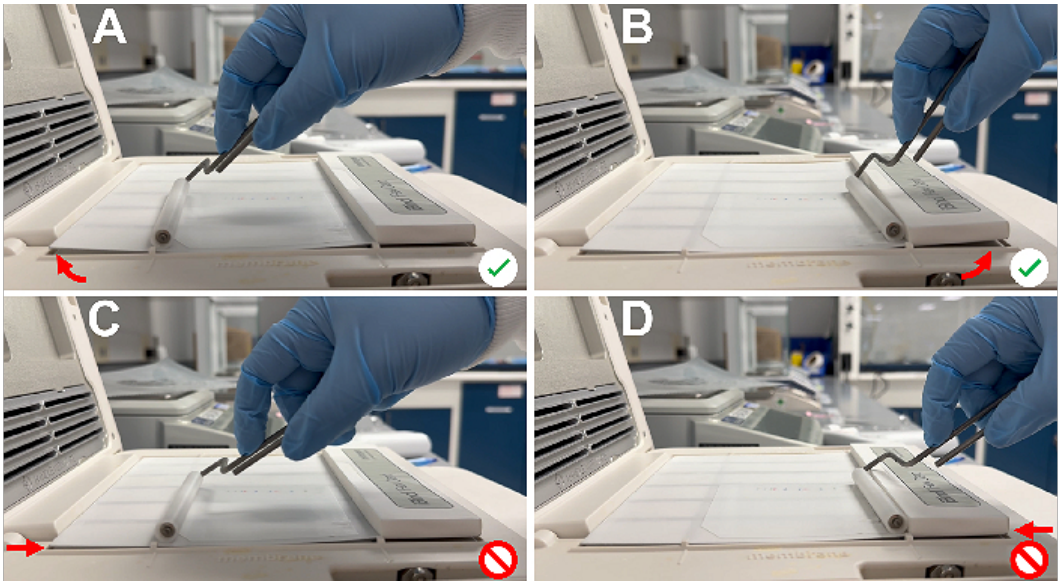


Figure 3 Firm rolling guidance. Firmly rolling the membrane on the iBind™ Flex Card ensures good contact and removes any air bubbles that affect immunoprocessing of the blot. After placing the membrane protein-side down on the iBind™ Flex Card, firmly roll the membrane on the card with a Blotting Roller to remove any air bubbles. (A and B) When firmly rolling the membrane, the iBind™ Flex Card will dip down in the membrane area, causing the top and bottom of the card to lift. (C and D) If the membrane is not properly rolled, the top and bottom of the card will not lift. It is critical to ensure good contact between the membrane and the iBind™ Flex Card. Dead bands and fuzziness can occur if there is poor contact between the membrane and card.

iBind™ Flex Card

The iBind™ Flex Card is a unique glass fiber matrix optimized for homogenous flow of immunodetection reagents.

The card consists of a Flow Region and a Stack. Solutions in the well inserts are released from the wells, and wicked towards the Stack via SLF.

IMPORTANT!

- **iBind™ Flex Cards are single use only. Discard card after use.**
- **Do not bend or crease the iBind™ Flex Cards. Bends, creases, or prominent wrinkles can result in poor immunodetection. Acceptable and unacceptable card conditions are shown in the figure below.**

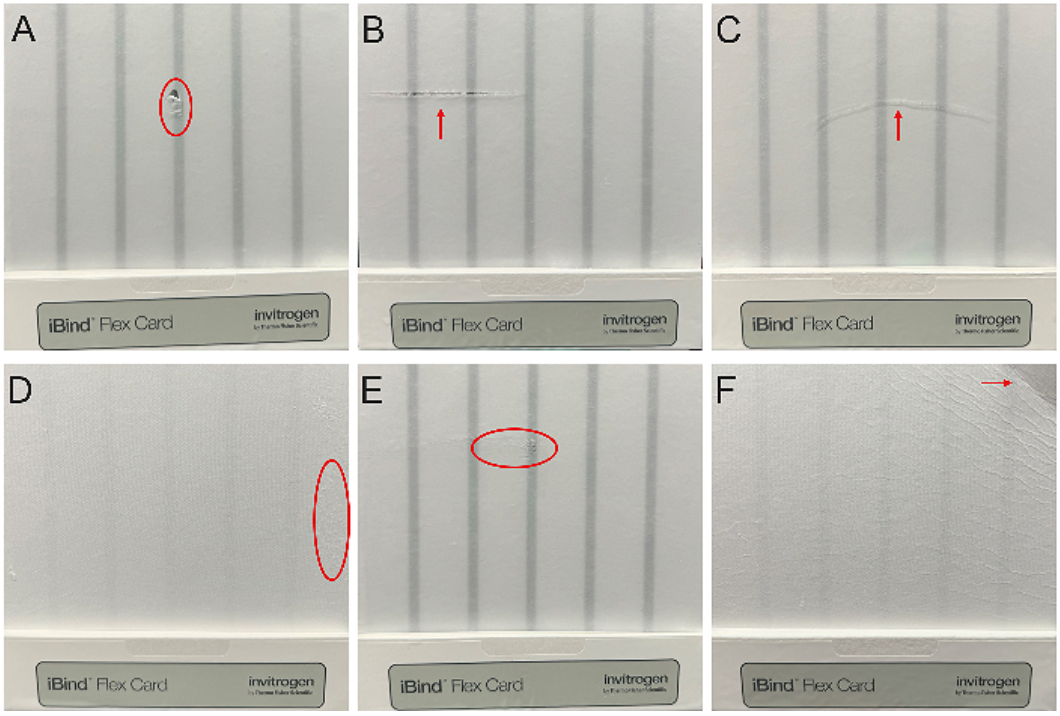


Figure 4 Examples of common iBind™ Flex Card mishandling. iBind™ Flex Cards should be handled by the stack to prevent damage prior to processing. Damage to the card can be caused by lab tools such as tweezers, rollers, and pipettes. Damage may also happen from mishandling of the card by the user. Using damaged cards will result in poor immunodetection. Figures of mishandling include: (A) Tweezer damage during readjustment of membrane; (B) Blot Roller damage due to excessive rolling; (C) Pipette damage during wetting of the card; (D) Damage due to rubbing of the card; (E) Blot Roller damage due to incorrect rolling; (F) Bending and wrinkles due to incorrect handling of the card.

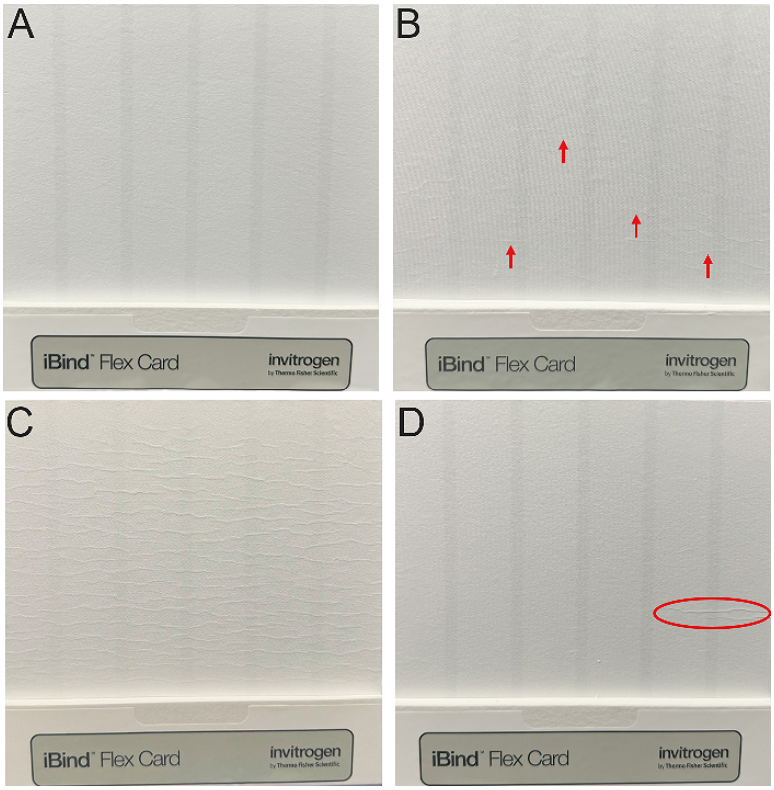
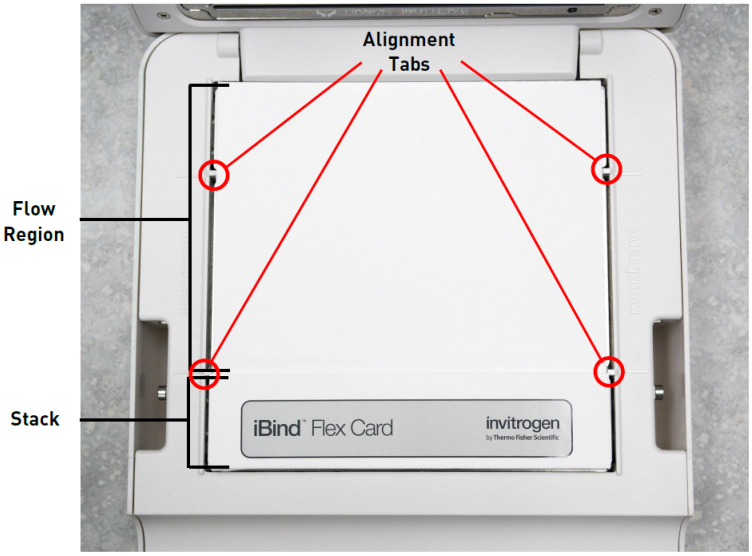


Figure 5 Acceptable and unacceptable conditions of iBind™ Flex Cards. iBind™ Flex Cards should be inspected for cracks, creases, and prominent wrinkles before using. Cards in unacceptable condition should not be used. Cards with few or no wrinkles are acceptable to use. Cards with many wrinkles, prominent wrinkles, or large fiber clumps should not be used. Figures of acceptable and unacceptable cards include: (A) Acceptable condition of card; (B) Acceptable card despite several minor wrinkles; (C) Unacceptable card due to excessive wrinkles; (D) Unacceptable card due to prominent wrinkles.



The iBind™ Flex Card is placed on the iBind™ Flex Western Device so that it fits between the alignment tabs with the stack facing the front of the device.

When the flow region is wet with solution, lines appear on the iBind™ Flex Card to assist in alignment of mini-sized or vertically cut strip membranes with lanes.

Procedural overview

General guidelines

CAUTION! Exercise care when closing the lid of the iBind™ Flex device to avoid catching fingers.

IMPORTANT! Ensure the membrane is placed **protein-side down** and firmly rolled on the iBind™ Flex Card to maintain good contact. Ensure the wells are not positioned over the membrane when the lid of the device is closed.

IMPORTANT! Handle iBind™ Flex Cards with care as bent, creased, or prominently wrinkled cards can result in poor immunodetection. See Figure 5 for a more detailed description of acceptable card conditions.

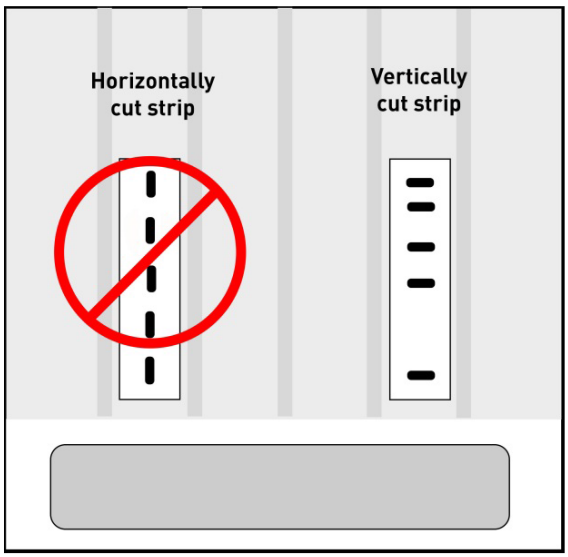
- Wear the proper protective equipment (gloves, laboratory coat, eye protection) when performing experiments.
- Handle well inserts with care, and keep them stored in the drawer of the iBind™ Flex device when not in use.
- If you mark your membrane(s) with ink, mark the membrane(s) near the low molecular weight region.
- The iBind™ Flex device is compatible with multiplexing when using iBind™ Flex Midi, Mini, or Multi-Strip inserts. See “Multiplexing antibodies” on page 25 for more details.
- When firmly rolling, the iBind™ Flex Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. See Figure 3 for additional details.
- Do not move the iBind™ Flex device or open the lid until the well(s) in row 4 are completely empty (2.5 hours or longer).

  Note: Membrane(s) can be left in the iBind™ Flex device overnight if desired.
- The 1X iBind™ Flex Solution is used for chemiluminescent detection, while 1X iBind™ Flex FD Solution is used for fluorescent detection.
- For best results, use final primary antibody concentration equal to 2X the manufacturer's recommended dilution (for example, use a 1:500 dilution if a 1:1,000 dilution is recommended).
- For best results, use a final secondary antibody concentration equal to 10X the manufacturer's recommended dilution (for example, use a 1:8,000 dilution if a 1:80,000 dilution is recommended).

- iBind™ Flex is compatible with conjugated primary antibodies where no secondary antibody is needed. See “Add solutions to wells” on page 29, page 37, and page 54 for additional details.
- The iBind™ Flex device can be used to reprobe stripped blots. It is not recommended to strip the blot using the iBind™ Flex device.

Guidelines for vertically cut membrane strips

- When using the iBind™ Flex Multi-Strip Insert, do not perform antibody binding on horizontally cut membrane strips.



- Vertically cut membrane strip should not exceed 1 inch (2.54 cm) in width.
  If using mini or midi protein gels from Thermo Fisher Scientific, refer to the following table for the number of sample lanes that can be accommodated in each vertically cut membrane strip.

Gel type	Sample lanes/vertically cut strip
10-well mini gel	3 lanes
12-well mini gel	4 lanes
15-well mini gel	5 lanes
17-well mini gel	6 lanes
20-well midi gel	4 lanes
26-well midi gel	5 lanes

Chemiluminescent detection procedure

Experimental overview

Use the following protocol when using the iBind™ Flex Western System with chemiluminescent detection protocols.

Step	Action	Page
1	Prepare 1X iBind™ Flex Solution	"Prepare 1X iBind™ Flex Solution" on page 20
2	Prepare membrane(s)	"Prepare membrane(s)" on page 21
3	Prepare diluted antibody solutions	"Prepare antibody solutions" on page 22
4	Perform antibody binding using: • Midi Insert • Mini Insert • Multi-Strip Insert	"Prepare the iBind™ Flex Card for the Midi Insert" on page 26 "Prepare the iBind™ Flex Card for the Mini Insert" on page 31 "Prepare the iBind™ Flex Card for the Multi-Strip Insert" on page 35
5	Perform detection	

Prepare 1X iBind™ Flex Solution

The 1X iBind™ Flex Solution is used for blocking, diluting antibodies, washing, and wetting the iBind™ Flex Card. Prepare the necessary volume of 1X iBind™ Flex Solution based on the tables below.

Table 1 Volume of 1X iBind™ Flex Solution needed for each step

Step	Midi	Mini		Vertically cut strips					
	Blot 1	Blot 1	Blot 2	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
Block membrane	10 mL	5 mL	5 mL	10 mL					
Wet card	10 mL	10 mL		10 mL					
Wet membrane region	2 mL	1 mL	1 mL	2 mL					
Primary antibody diluent	4 mL	2 mL	2 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL
First wash	4 mL	2 mL	2 mL	2 mL			2 mL		
Secondary antibody diluent	4 mL	2 mL	2 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL
Final wash	12 mL	6 mL	6 mL	6 mL			6 mL		
Total volume	**46 mL**	**46 mL**		**46.4 mL**					

For example, if 50 mL of iBind™ Flex Solution is needed, prepare solution according to the table below.

Reagent	Volume
iBind™ Flex 5X Buffer	10 mL
iBind™ Flex 100X Additive	500 µL
Distilled water	39.5 mL
Total volume	**50 mL**

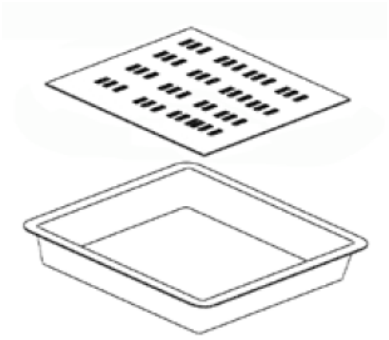
Prepare membrane(s)

It is recommended to proceed with blocking and iBind™ processing immediately after transfer. If storage of membranes is required prior to processing, store membranes in distilled water or dry.

Block membranes only with 1X iBind™ Flex Solution. Use of other blockers may interfere with iBind™ processing.

Before performing the antibody binding, prepare the membrane as follows:

- Pre-activate PVDF membranes in 100% methanol, and rinse in distilled water.
- Immerse the blotted membrane (**protein-side up**) in 1X iBind™ Flex Solution. Use 10–20 mL for midi-sized membranes or for vertically cut strips, or 5–10 mL for each mini-sized membrane based on the size of the incubation tray. Ensure blot is fully submerged in solution. Incubate for 2–10 minutes with or without shaking.



Prepare antibody solutions

A different primary antibody can be used in each lane (well 1) when performing detection using iBind™ Flex Multi-Strip or Mini Inserts.

If performing detection with different primary antibodies in each lane, use the appropriate secondary antibody in the corresponding lane (well 3).

Dilute primary and secondary antibodies in the appropriate volume of 1X iBind™ Flex Solution depending on the membrane size and according to the tables below.

Prepare primary antibody solution			
Component	**Midi blot**	**Mini blot**	**Vertical strip**
1X iBind™ Flex Solution	4 mL	2 mL	0.7 mL
1° Antibody	Dilute the primary antibody to 2X the manufacturer's recommended dilution[1] (for example, use a 1:500 dilution if a 1:1,000 dilution is recommended).		
Prepare secondary antibody solution			
Component	**Midi blot**	**Mini blot**	**Vertical strip**
1X iBind™ Flex Solution	4 mL	2 mL	0.7 mL
2° Antibody	Dilute the secondary antibody to 10X the manufacturer's recommended dilution[1] (for example, use a 1:8,000 dilution if a 1:80,000 dilution is recommended).		

[1] Recommended starting dilutions. Antibody dilutions can be adjusted to achieve the desired signal.

Note: If using the chemiluminescent procedure, go to page 26 for a Midi Insert, page 31 for a Mini Insert, or page 35 for a Multi-Strip Insert to begin preparing the iBind™ Flex Card.

Fluorescent detection procedure

Experimental overview

Use the following protocol when using the iBind™ Flex Western System in conjunction with fluorescent detection.

Step	Action	Page
1	Prepare 1X iBind™ Flex FD Solution	"Prepare 1X iBind™ Flex FD Solution" on page 24
2	Prepare membrane(s)	"Prepare membrane(s)" on page 25
3	Prepare diluted antibody solutions	"Prepare antibody solutions" on page 26
4	Perform antibody binding using: • Midi insert • Mini insert • Multi-strip insert	"Prepare the iBind™ Flex Card for the Midi Insert" on page 26 "Prepare the iBind™ Flex Card for the Mini Insert" on page 31 "Prepare the iBind™ Flex Card for the Multi-Strip Insert" on page 35
5	Perform detection	

Prepare 1X iBind™ Flex FD Solution

The 1X iBind™ FD Flex Solution is used for blocking, diluting antibodies, washing, and wetting the iBind™ Flex Card.

- The Standard 1X iBind™ Flex FD Solution is recommended for use with most primary antibodies.
- Use the Optional 1X iBind™ Flex FD Solution only if initial results give low sensitivity or high background.

Prepare the necessary volume of 1X iBind™ Flex FD Solution based on the tables below.

Table 2 Volume of iBind™ Flex FD Solution needed for each step

Step	Midi	Mini		Vertically cut strips					
	Blot 1	Blot 1	Blot 2	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
Block membrane	10 mL	5 mL	5 mL	10 mL					
Wet card	10 mL	10 mL		10 mL					
Wet membrane region	2 mL	1 mL	1 mL	2 mL					
Primary antibody diluent	4 mL	2 mL	2 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL
First wash	4 mL	2 mL	2 mL	2 mL			2 mL		
Secondary antibody diluent	4 mL	2 mL	2 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL	0.7 mL
Final wash	12 mL	6 mL	6 mL	6 mL			6 mL		
Total volume	**46 mL**	**46 mL**		**46.4 mL**					

For example, if 50 mL of iBind™ Flex FD Solution is needed, prepare solution according to the table below:

Reagent	Volume	
	Standard	Optional
iBind™ Flex FD 5X Buffer	10 mL	2.5 mL
iBind™ Flex 100X Additive	125 µL	500 µL
Distilled water	39.9 mL	47 mL
Total	**50 mL**	**50 mL**

Prepare membrane(s)

It is recommended to proceed with blocking and iBind™ processing immediately after transfer. If storage of membranes is required prior to processing, store membranes in distilled water or dry.

Block membranes with only 1X iBind™ Flex FD Solution. Use of other blockers may interfere with iBind™ processing.

- Pre-activate PVDF membranes in 100% methanol, and rinse in distilled water.
- Immerse the blotted membrane (**protein-side up**) in 1X iBind™ Flex FD Solution. Use 10–20 mL for midi-sized membranes or for vertically cut strips, or 5–10 mL for each mini-sized membrane based on the size of the incubation tray. Ensure blot is fully submerged in solution. Incubate for 2–10 minutes, with or without shaking.

Multiplexing antibodies

Antibodies can be multiplexed to perform detection when using iBind™ Flex Midi, Mini, or Multi-Strip Inserts.

- **Primary antibodies**: For each midi blot, mini blot, or vertically cut strip, prepare appropriate primary antibodies together in a single tube of iBind™ Flex FD Solution. All primary antibodies should be diluted according to the recommendations outlined in the table below.
- **Secondary antibodies**: For each midi blot, mini blot, or vertically cut strip, prepare appropriate secondary antibodies together in a single tube of iBind™ Flex FD Solution. All secondary antibodies should be diluted according to the recommendations outlined in the table below. When detecting several targets of the same species the same concentration of secondary can be used as when detecting one target. There is no need to increase the concentration for each additional target.
- **IMPORTANT!** Consider cross-reactivity of secondary antibodies when multiplexing (for example, rabbit anti-goat IgG and goat anti-mouse IgG are likely to cross-react).
- Load multiplexed antibodies into the device as normal.

Prepare antibody solutions

Dilute primary and secondary antibodies in the appropriate volume of 1X iBind Flex FD Solution depending on the membrane size and according to the tables below.

Prepare primary antibody solution			
Component	**Midi blot**	**Mini blot**	**Vertical strip**
1X iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
1° Antibody	Dilute the primary antibody to 2X the manufacturer's recommended dilution[1] (for example, use a 1:500 dilution if a 1:1,000 dilution is recommended).		
Prepare secondary antibody solution			
Component	**Midi blot**	**Mini blot**	**Vertical strip**
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
2° Antibody	Dilute the secondary antibody to 10X the manufacturer's recommended dilution[1] (for example, use a 1:8,000 dilution if a 1:80,000 dilution is recommended).		

[1] Recommended starting dilutions. Antibody dilutions can be adjusted to achieve the desired signal.

[2] SDS is added to a final concentration of 0.05% to reduce background signal, particularly when using PVDF membranes, or fluorophore-conjugated secondary antibodies.

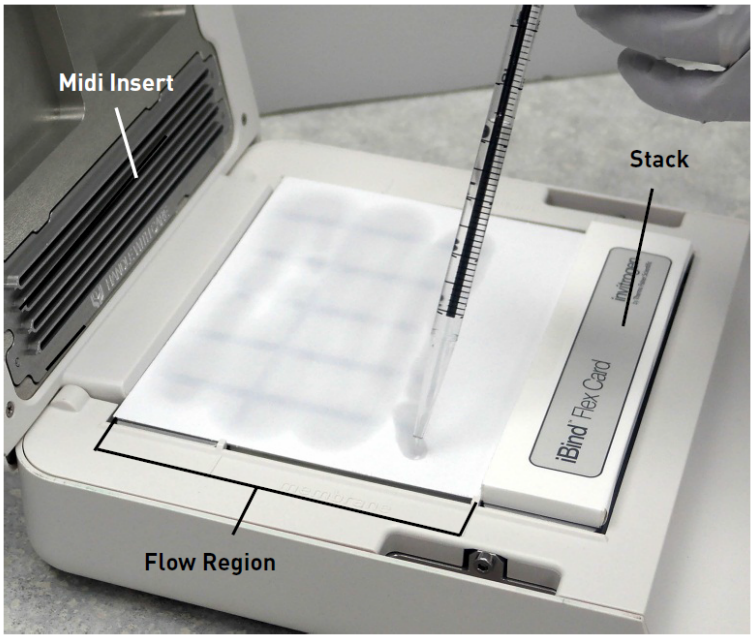
Note: If using the fluorescent procedure, go to page 26 for a Midi Insert, page 31 for a Mini Insert, or page 35 for a Multi-Strip Insert to begin preparing the iBind™ Flex Card.

Use the iBind™ Flex device with the Midi Insert

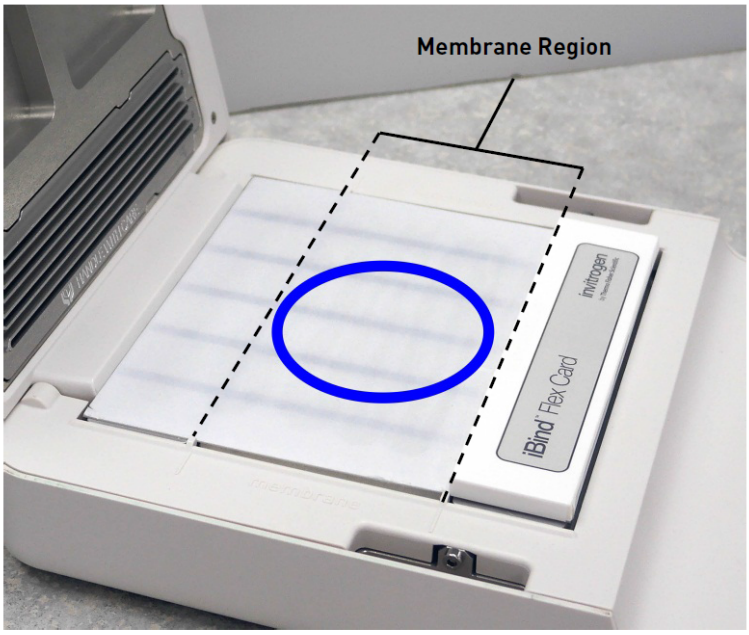
Prepare the iBind™ Flex Card for the Midi Insert

1. Open the lid of the iBind™ Flex device.
2. Verify the Midi Insert is inserted in the iBind™ Flex device.
3. Open the packaging and remove the iBind™ Flex Card, grasping the card by the stack.
4. Inspect the condition of the iBind™ Flex Card. If cracks, creases, or prominent wrinkles are observed, do not use the card. See "iBind™ Flex Card" on page 14 for a more detailed description of acceptable card conditions.
5. Place the iBind™ Flex Card on the Stage.

6. Pipette 10 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution evenly across the Flow Region.

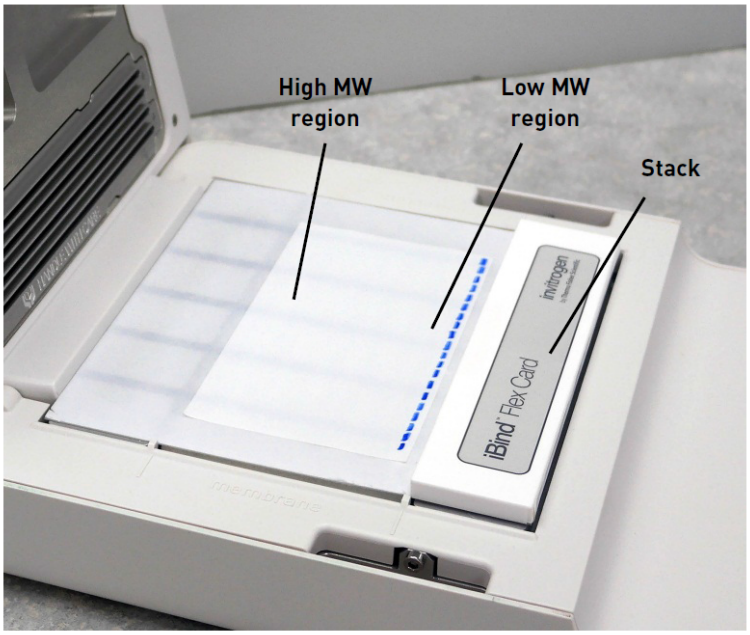


7. Pipette 2 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution so that it pools at the center of the membrane region on the iBind™ Flex Card.



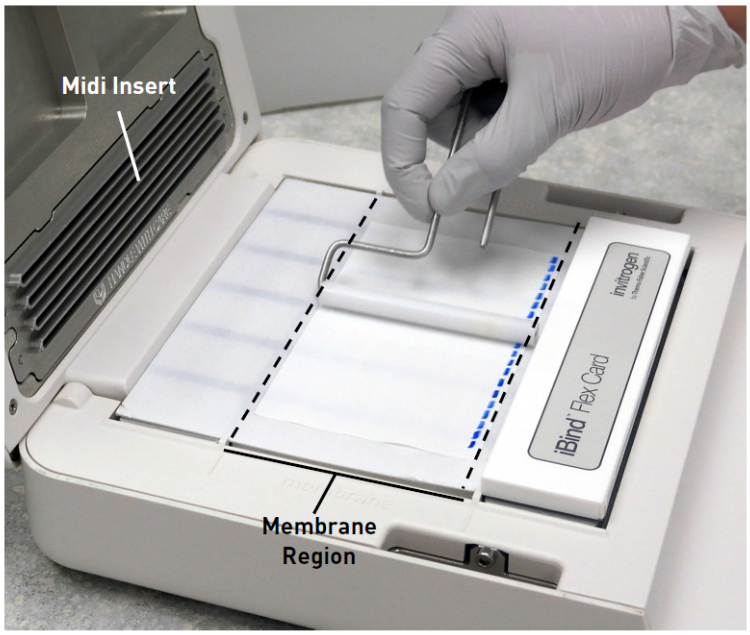
Place membrane on the iBind™ Flex Card

1. Place the membrane on top of the pooled solution with the **protein-side down**, and the low molecular weight protein region closest to the Stack. **Do not allow the membrane to come in contact with the stack.**



2. 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. See Figure 3 for additional rolling details.



3. Make sure the membrane is within the boundaries of the membrane region. **Ensure no part of the membrane is directly under the Midi Insert.**

4. Lower the lid of the iBind™ Flex device and close the latch handle to lock the lid.

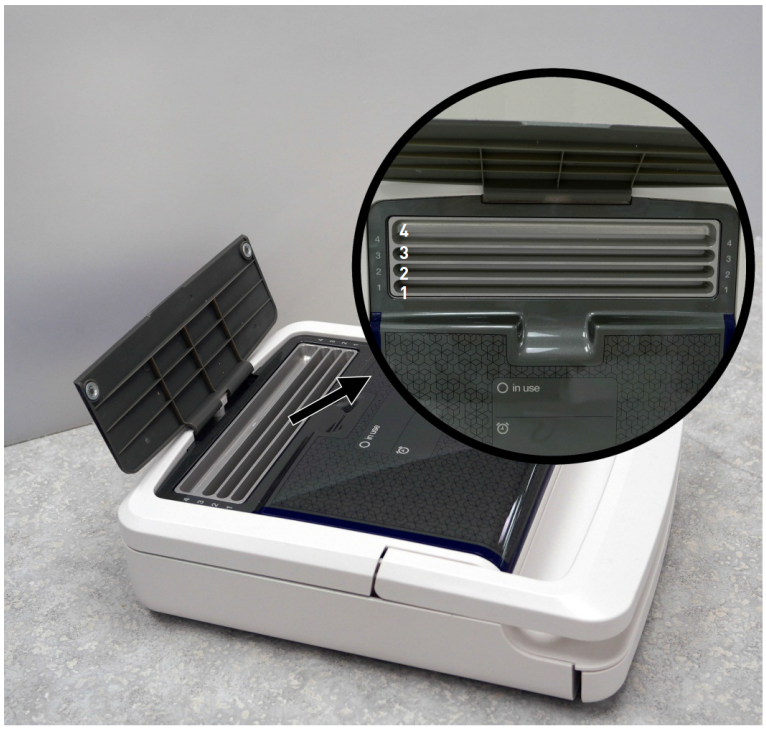
Add solutions to wells

1. Open the Well Cover and add solutions sequentially to the iBind™ Flex wells **starting with row 1** (do not exceed the total volume/well).

Row	Solution	Volume/Well
1	Diluted primary antibody[1,2]	4 mL
2	1X iBind™ Flex/iBind™ Flex FD Solution	4 mL
3	Diluted secondary antibody[2]	4 mL
4	1X iBind™ Flex/iBind™ Flex FD Solution	12 mL

[1] Antibodies can be multiplexed for fluorescent detection. (see "Multiplexing antibodies" on page 25 for details).

[2] **Conjugated Primary Antibody Loading Procedure**: When using a conjugated primary antibody and no secondary antibody, the diluted primary antibody solution is added to well 1. Diluted secondary antibody (well 3) is replaced with 1X iBind™ Flex/iBind™ Flex FD Solution. **Alternative Conjugated Primary Antibody Loading Procedure**: For faster time to results, add diluted primary antibody to well 1, leave wells 2 and 3 empty, and add 1X iBind™ Flex/ iBind™ Flex FD Solution to well 4. This procedure will save time (approximately 60 min.) but may result in higher background.



2. Close the Well Cover and write the start time of incubation on the lid of the iBind™ Flex device.

3. Incubate 2.5 hours or longer.

Note: Membranes can be left in the iBind™ Flex device overnight, but perform detection as soon as possible to avoid loss of sensitivity.

---

4. Open the Well Cover to verify that row 4 is completely empty (indicating that the run is over) before releasing the latch handle and opening the lid.

5. After incubation, rinse the membrane twice in 20 mL of distilled water for 2 minutes and proceed to the preferred detection protocol.

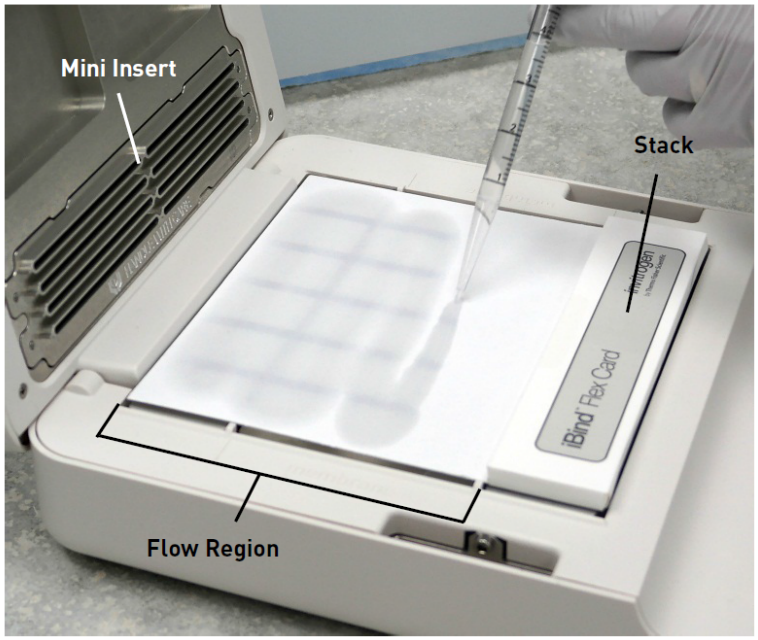
6. Discard the iBind™ Flex Card after use.

Note: Blots processed with iBind™ Flex device can be stripped and reprobed. After the stripping protocol, the blot can be reprobed with a new iBind™ Flex processing run as normal. However, many stripping and reprobing reagents decrease signal of the second target, therefore antibody optimization may be necessary. It is not recommended to strip the blot in the iBind™ Flex device.

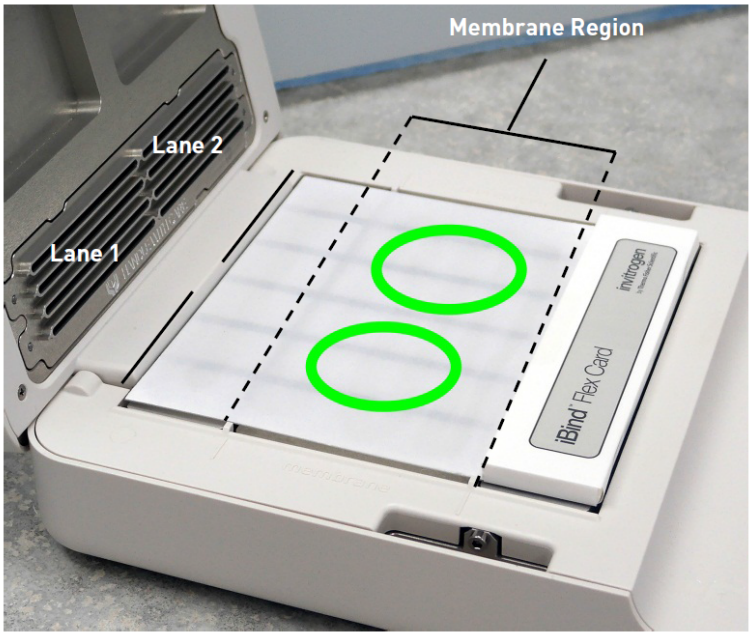
Use the iBind™ Flex device with the Mini Insert

Prepare the iBind™ Flex Card for the Mini Insert

1. Open the lid of the iBind™ Flex device.
2. Verify the Mini Insert is inserted in the iBind™ Flex device.
3. Open the packaging and remove the iBind™ Flex Card, grasping the card by the stack.
4. Inspect the condition of the iBind™ Flex Card. If cracks, creases, or prominent wrinkles are observed, do not use the card. See "iBind™ Flex Card" on page 14 for a more detailed description of acceptable card conditions.
5. Place the iBind™ Flex Card on the Stage.
6. Pipette 10 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution evenly across the Flow Region.

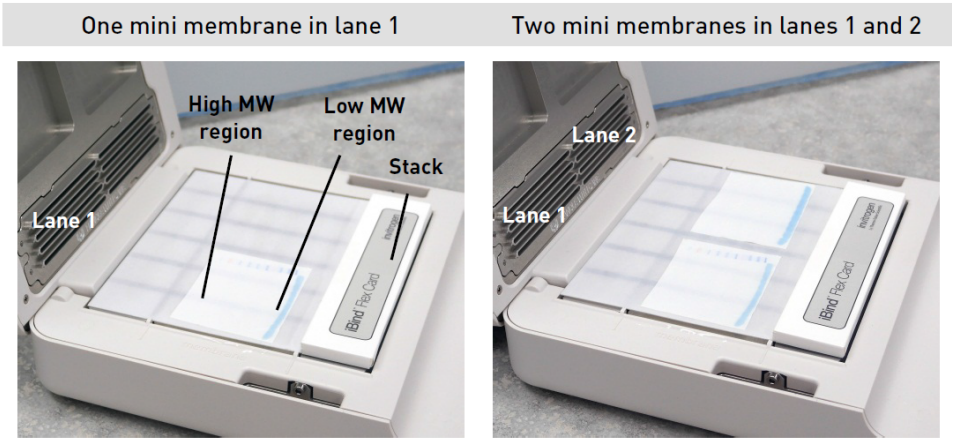


7. Pipette 1 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution in each lane to be used for a membrane so that it pools at the membrane region on the iBind™ Flex Card.



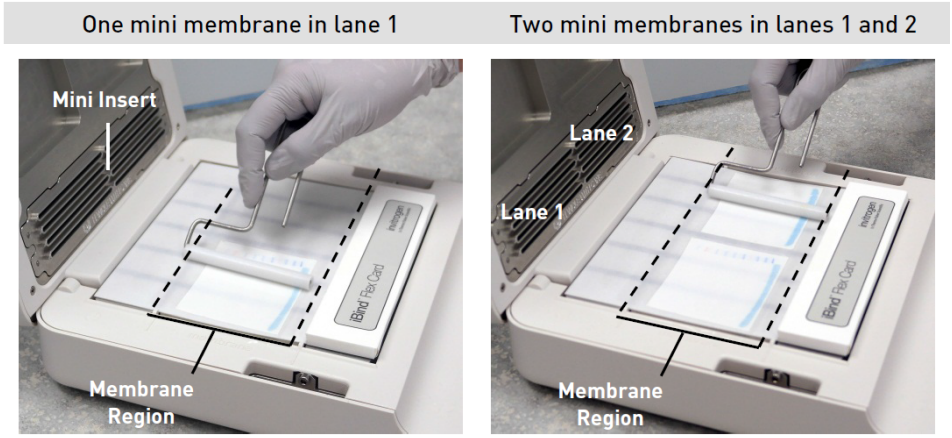
Place membrane on the iBind™ Flex Card

1. Place the membrane on top of the pooled solution with the **protein-side down**, and the low molecular weight protein region closest to the Stack. **Do not allow the membrane to come in contact with the stack.**



2. 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. See Figure 3 for additional rolling details.



3. Ensure the membrane(s) are aligned with the lane(s) and within the boundaries of the membrane region. **Ensure no part of the membrane is directly under the Mini Insert.**

4. Lower the lid of the iBind™ Flex device and close the latch handle to lock the lid.

Add solutions to wells

1. Open the Well Cover and add solutions sequentially to the iBind™ Flex Wells **starting with row 1** (do not exceed the total volume/well).

Row	Solution[1]	Volume/Well
1	Diluted primary antibody[2,3]	2 mL
2	1X iBind™ Flex/iBind™ Flex FD Solution	2 mL
3	Diluted secondary antibody[3]	2 mL
4	1X iBind™ Flex/iBind™ Flex FD Solution	6 mL

[1] If only one membrane is being probed, add water to the unused lane at the given volume for each row.

[2] A different antibody can be used for each lane. Antibodies can also be multiplexed for fluorescent detection (see “Multiplexing antibodies” on page 25 for details).

[3] **Conjugated Primary Antibody Loading Procedure**: When using a conjugated primary antibody and no secondary antibody, the diluted primary antibody solution is added to well 1. Diluted secondary antibody (well 3) is replaced with 1X iBind™ Flex/iBind™ Flex FD Solution. **Alternative Conjugated Primary Antibody Loading Procedure**: For faster time to results, add diluted primary antibody to well 1, leave wells 2 and 3 empty, and add 1X iBind™ Flex/ iBind™ Flex FD Solution to well 4. This procedure will save time (approximately 60 min.) but may result in higher background.

2. Close the Well Cover and write the start time of incubation on the lid of the iBind™ Flex device.

3. Incubate 2.5 hours or longer.

Note: Membranes can be left in the iBind™ Flex device overnight, but perform detection as soon as possible to avoid loss of sensitivity.

4. Open the Well Cover to verify that the well(s) in row 4 are completely empty (indicating that the run is over) before releasing the latch handle and opening the lid.

5. After incubation, rinse the membrane twice in 20 mL of distilled water for 2 minutes, then proceed to the preferred detection protocol.

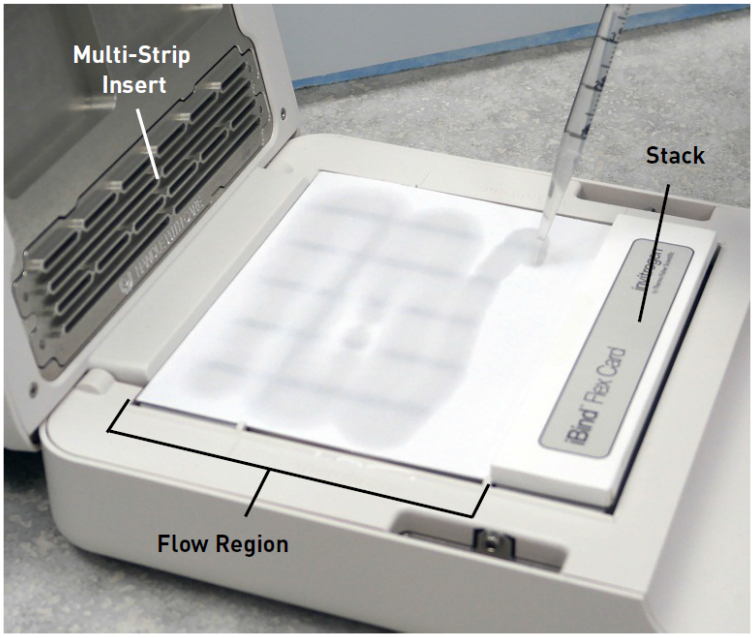
6. Discard the iBind™ Flex Card after use.

Note: Blots processed with the iBind™ Flex device can be stripped and reprobed. After the stripping protocol, the blot can be reprobed with a new iBind™ Flex processing run as normal. However, many stripping and reprobing reagents decrease signal of the second target, therefore antibody optimization may be necessary. It is not recommended to strip the blot in the iBind™ Flex device.

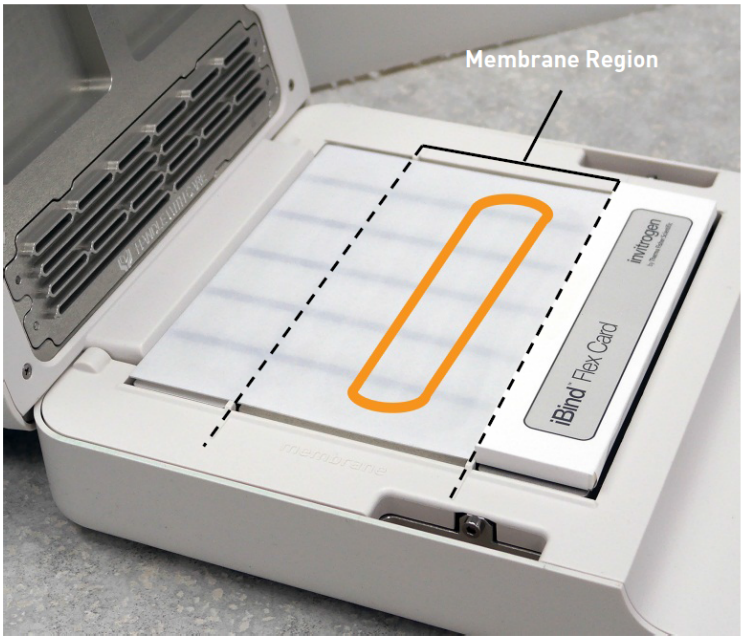
Use the iBind™ Flex device with the Multi-Strip Insert

Prepare the iBind™ Flex Card for the Multi-Strip Insert

1. Open the lid of the iBind™ Flex device.
2. Verify the Multi-Strip Insert is inserted in the iBind™ Flex device.
3. Open the packaging and remove the iBind™ Flex Card, grasping the card by the stack.
4. Inspect the condition of the iBind™ Flex Card. If cracks, creases, or prominent wrinkles are observed, do not use the card. See “iBind™ Flex Card” on page 14 for a more detailed description of acceptable card conditions.
5. Place the iBind™ Flex Card on the Stage.
6. Pipette 10 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution evenly across the Flow Region.



7. Pipette 2 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution so that it pools along the center of the membrane region on the iBind™ Flex Card.

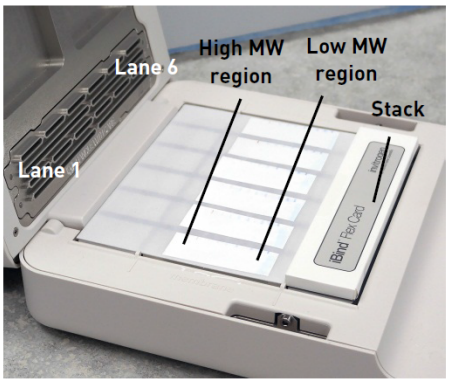


Place membrane on the iBind™ Flex Card

1. Place the membrane on top of the pooled solution with the **protein-side down**, and the low molecular weight protein region closest to the Stack. **Do not allow the membrane to come in contact with the stack.**

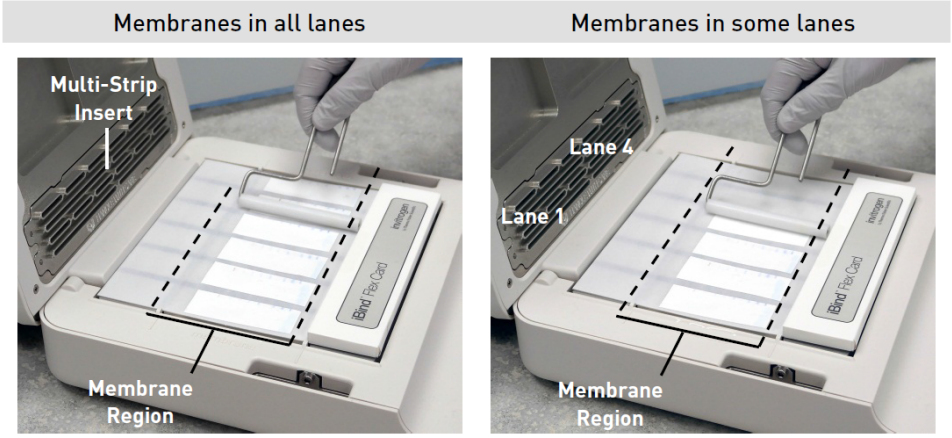
Note: Vertically cut membrane strips should not exceed 1 inch (2.54 cm) in width. Do not perform antibody binding on horizontally cut membrane strips.

Membranes in all lanes	Membranes in some lanes



2. 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. See Figure 3 for additional rolling details.



3. Ensure the membrane(s) are aligned with the lane(s) and within the boundaries of the membrane region. **No part of the membrane should be directly under the Multi-Strip Insert.**

4. Lower the lid of the iBind™ Flex Western Device and close the latch handle to lock the lid.

Add solutions to wells

1. Open the Well Cover and add solutions sequentially to the iBind™ Flex Wells **starting with row 1** (do not exceed the total volume/well).

Row	Solution[1]	Volume/Well
1	Diluted primary antibody[2,3]	0.7 mL
2	1X iBind™ Flex/ iBind™ Flex FD Solution	2 mL
3	Diluted secondary antibody[3]	0.7 mL
4	1X iBind™ Flex/ iBind™ Flex FD Solution	6 mL

[1] If not all lanes are being used to process vertically cut strips, add water at the given volume for each row in the unused lane(s).

[2] A different antibody can be used for each lane. Antibodies can also be multiplexed for fluorescent detection (see “Multiplexing antibodies” on page 25 for details).

[3] **Conjugated Primary Antibody Loading Procedure**: When using a conjugated primary antibody and no secondary antibody, the diluted primary antibody solution is added to well 1. Diluted secondary antibody (well 3) is replaced with 1X iBind™ Flex/iBind™ Flex FD Solution. **Alternative Conjugated Primary Antibody Loading Procedure**: For faster time to results, add diluted primary antibody to well 1, leave wells 2 and 3 empty, and add 1X iBind™ Flex/ iBind™ Flex FD Solution to well 4. This procedure will save time (approximately 60 min.) but may result in higher background.

2. Close the Well Cover and write the start time of incubation on the lid of the iBind™ Flex Western device.

3. Incubate 2.5 hours or longer.

Note: Membranes can be left in the iBind™ Flex Western Device overnight, but perform detection as soon as possible to avoid loss of sensitivity.

---

4. Open the Well Cover to verify that the well(s) in row 4 are completely empty (indicating that the run is over) before releasing the latch handle and opening the lid.

5. After incubation, rinse the membrane twice in 20 mL of distilled water for 2 minutes, then proceed to the preferred detection protocol.

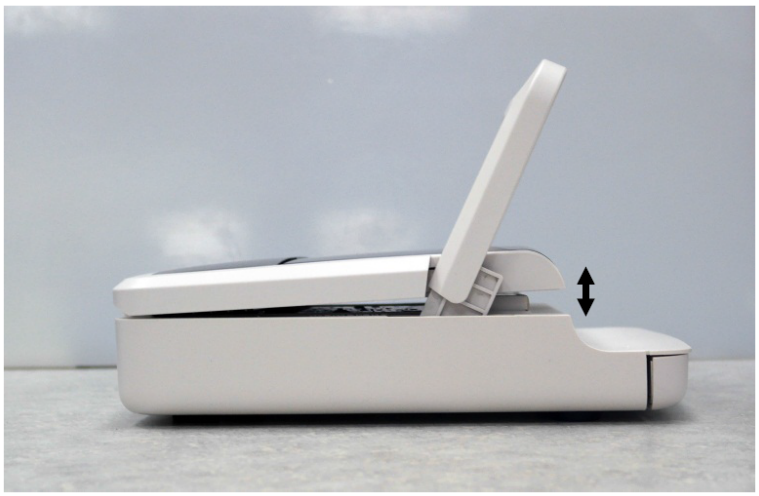
6. Discard iBind™ Flex Card after use.

Note: Blots processed with the iBind™ Flex device can be stripped and reprobed. After the stripping protocol, the blot can be reprobed with a new iBind™ Flex processing run as normal. However, many stripping and reprobing reagents decrease signal of the second target, therefore antibody optimization may be necessary. It is not recommended to strip the blot in the iBind™ Flex device.

Maintenance

General guidelines

- Rinse the iBind™ Flex well inserts under running water after each use and allow the well inserts to dry before additional usage.
- **Handle well inserts with care.**
- Store unused well inserts in the drawer in the iBind™ Flex Western Device.
- To maximize the life of the springs in iBind™ Flex Western Device, store the device with latch unlocked, and the lid open as shown below:



A Optimization and troubleshooting

Optimization

Antibody dilution optimization

After performing an initial chemiluminescent or fluorescent experiment, conditions can be optimized by varying the concentration of primary and secondary antibodies according to the following table.

Condition/Observation	Primary and secondary antibody concentrations	
	Primary	Secondary
Low signal	2X–5X the manufacturer's recommended dilution[1]	10X the manufacturer's recommended dilution
High background with strong signal	Manufacturer's recommended dilution	1X–10X the manufacturer's recommended dilution

[1] For example, use a 1:200–1:500 dilution if a 1:1,000 dilution is recommended.

Note: If needed, antibody concentrations can be adjusted outside of these recommendations to achieve the desired result.

iBind™ Flex Card condition

The condition of the iBind™ Flex Card is critical for optimal results. See the figure below for examples of both acceptable and unacceptable cards.

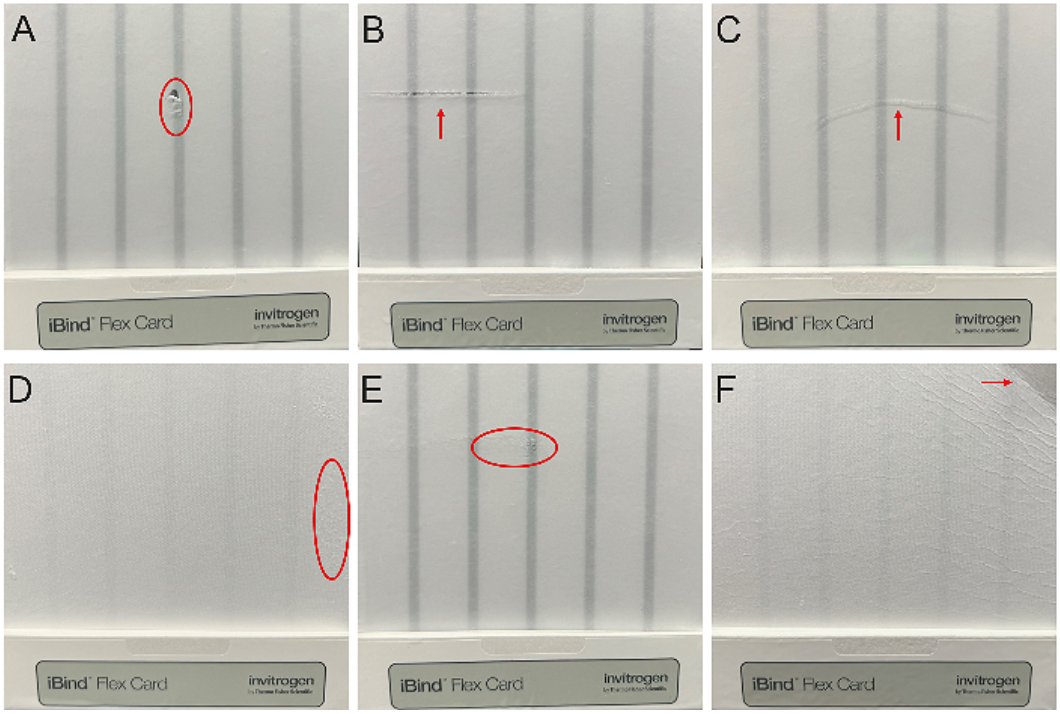


Figure 6 Examples of common iBind™ Flex Card mishandling. iBind™ Flex Cards should be handled by the stack to prevent damage prior to processing. Damage to the card can be caused by lab tools such as tweezers, rollers, and pipettes. Damage may also happen from mishandling of the card by the user. Using damaged cards will result in poor immunodetection. Figures of mishandling include: (A) Tweezer damage during readjustment of membrane; (B) Blot Roller damage due to excessive rolling; (C) Pipette damage during wetting of the card; (D) Damage due to rubbing of the card; (E) Blot Roller damage due to incorrect rolling; (F) Bending and wrinkles due to incorrect handling of the card.

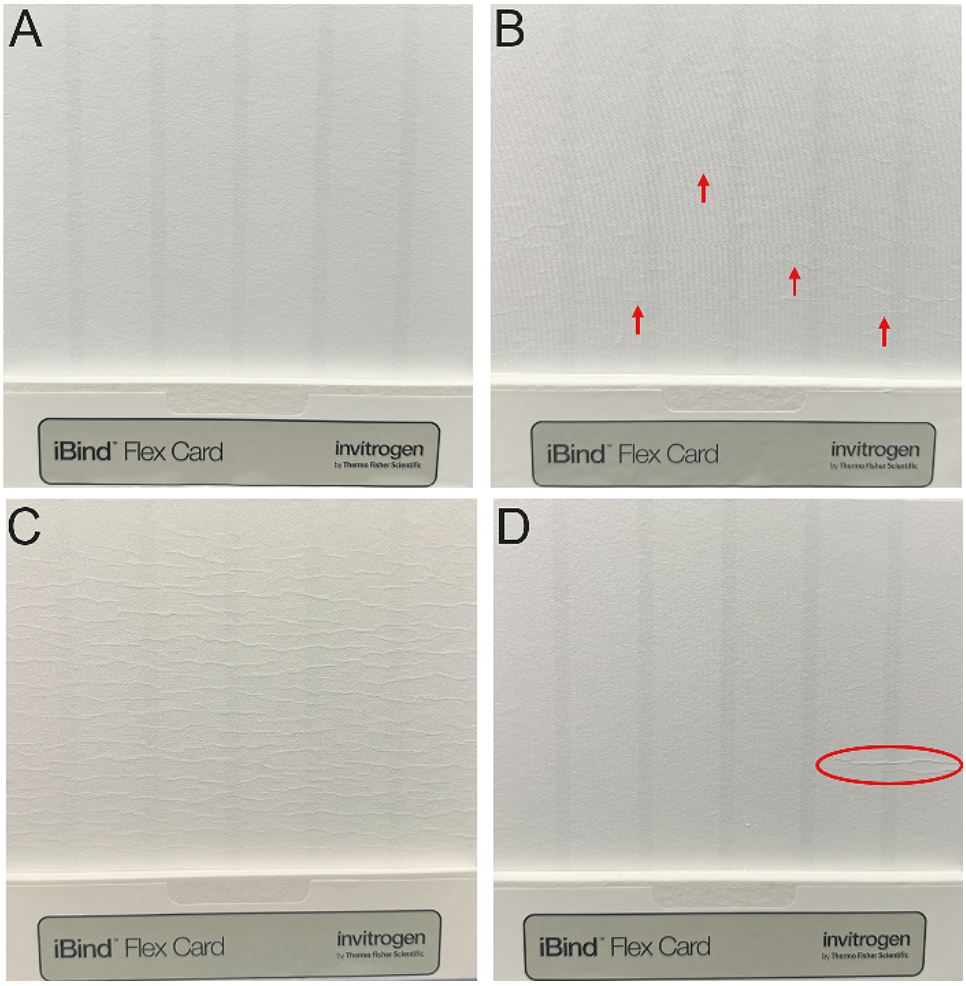


Figure 7 Acceptable and unacceptable conditions of iBind™ Flex Cards. iBind™ Flex Cards should be inspected for cracks, creases, and prominent wrinkles before using. Cards in unacceptable condition should not be used. Cards with few or no wrinkles are acceptable to use. Cards with many wrinkles, prominent wrinkles, or large fiber clumps should not be used. Figures of acceptable and unacceptable cards include: (A) Acceptable condition of card; (B) Acceptable card despite several minor wrinkles; (C) Unacceptable card due to excessive wrinkles; (D) Unacceptable card due to prominent wrinkles.

Firm rolling

Firm rolling is needed to ensure optimal results. When firmly rolling, the iBind™ Flex Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. The Blotting Roller is used to remove any air bubbles between the membrane and the iBind™ Flex Card.

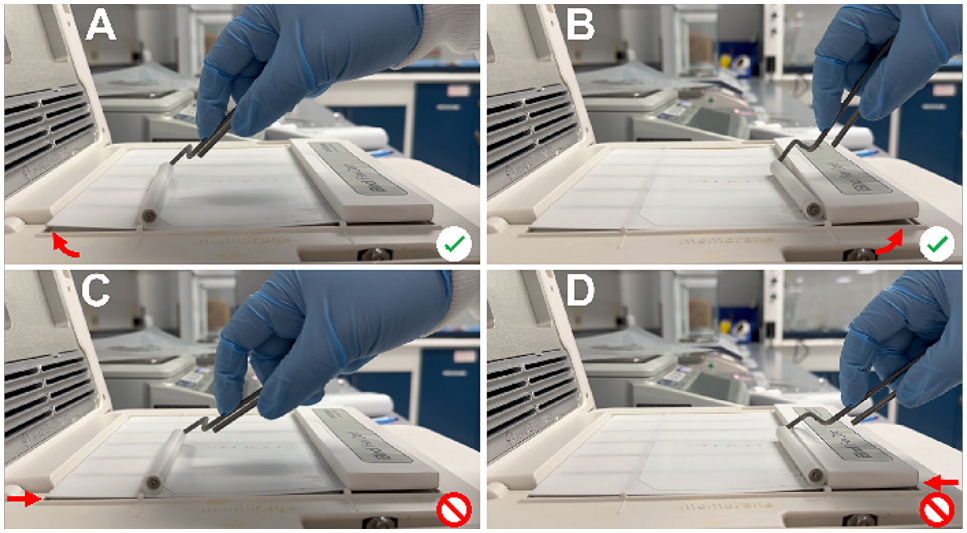


Figure 8 Firm rolling guidance. Firmly rolling the membrane on the iBind™ Flex Card ensures good contact and removes any air bubbles that affect immunoprocessing of the blot. After placing the membrane protein-side down on the iBind™ Flex Card, firmly roll the membrane on the card with a Blotting Roller to remove any air bubbles. (A and B) When firmly rolling the membrane, the iBind™ Flex Card will dip down in the membrane area, causing the top and bottom of the card to lift. (C and D) If the membrane is not properly rolled, the top and bottom of the card will not lift. It is critical to ensure good contact between the membrane and the iBind™ Flex Card. Dead bands and fuzziness can occur if there is poor contact between the membrane and card.

Troubleshooting the iBind™ Flex device

Observation	Possible Cause	Solution
Run times greater than 3 hours	iBind™ Flex Card was damaged.	Replace with a new card. Ensure card is free of prominent wrinkles, creases, and bends (see "iBind™ Flex Card" on page 14 for a more detailed description of acceptable card conditions). Ensure that rolling of the membrane on the card is limited to the area labeled "membrane".
	Initial over-wetting of the iBind™ Flex Card.	Only use the recommended volume of 1X iBind™ Flex/iBind™ Flex FD Solution when preparing the card. Avoid adding solution to the Stack.
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution.	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed ("Prepare 1X iBind™ Flex Solution" on page 20 or "Prepare 1X iBind™ Flex FD Solution" on page 24). It is not recommended to use non-iBind™ solutions (for example, blocking buffers, wash buffers, etc.).
	Improper blocking buffer was used.	The iBind™ Flex/iBind™ Flex FD Solution is used as a combined blocking, washing, and antibody diluent solution. The iBind™ Flex and iBind™ Flex FD solutions have specific viscosity and are optimized for sequential lateral flow. To avoid failure, it is not recommended to use other blockers. We cannot guarantee the performance with any other solutions.
High background	Membrane was not completely wet.	Follow instructions for pre-wetting the membrane (see "Prepare membrane(s)" on page 21 or "Prepare membrane(s)" on page 25). Use an incubation dish small enough to allow thorough coverage of the membrane to prevent drying out.
	Concentrated primary or secondary antibody was used.	Start by decreasing the secondary antibody concentration to 1X–10X until desired background intensity is achieved. If high background persists at 1X secondary antibody concentration, decrease the primary antibody concentration to 1X–2X.
	Ink was used to label membrane.	Any labeling of the membrane with ink should be limited to the low MW region of the blot.
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution.	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed ("Prepare 1X iBind™ Flex Solution" on page 20 or "Prepare 1X iBind™ Flex FD Solution" on page 24). It is not recommended to use non-iBind™ solutions (for example, blocking buffers, wash buffers, etc.).
	Solutions were improperly applied to iBind™ Flex Wells.	Add the appropriate solutions for each well in numerical order (see page 29 for Mini Inserts, page 33 for Midi Inserts, or page 37 for Multi-Strip Inserts).

(continued)

Observation	Possible Cause	Solution
High background	Blot was improperly placed on iBind™ Flex Card.	• Place the membrane in the designated Membrane Region on the iBind™ Flex Card. • Firmly roll the membrane on the iBind™ Flex Card to ensure good contact (see Figure 8). • Ensure the low MW regions are closest to the Stack. • Ensure the membrane does not contact the Stack.
	Card stack was wet prior to run.	Ensure that 10 mL of 1X iBind™ Flex/iBind™ Flex FD Solution is added to the flow region of the card. Avoid adding the solution to the Stack.
Nonspecific binding	Primary antibody was too concentrated.	Start with a 2X concentration of primary antibody. Further optimization by decreasing the primary antibody concentration may be necessary depending on the desired level of signal.
	Insufficient removal of SDS/weakly bound proteins from membrane after blotting.	Follow instructions for membrane preparation before immunodetection ("Prepare 1X iBind™ Flex Solution" on page 20, "Prepare 1X iBind™ Flex FD Solution" on page 24).
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution.	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed ("Prepare 1X iBind™ Flex Solution" on page 20, "Prepare 1X iBind™ Flex FD Solution" on page 24).
Weak or no signal	Membrane was not completely wet.	Follow instructions for pre-wetting the membrane (see "Prepare membrane(s)" on page 21 or "Prepare membrane(s)" on page 25). Use an incubation dish small enough to allow thorough coverage of the membrane to prevent drying out.
	Primary or secondary antibody concentration was too low.	Start with a 10X concentration of secondary antibody. If weak or no signal is still observed, adjust the primary antibody concentration, starting with a 2X concentration of primary antibody and increasing to 2X-5X depending on the desired signal.
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution.	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed ("Prepare 1X iBind™ Flex Solution" on page 20 or "Prepare 1X iBind™ Flex FD Solution" on page 24). It is not recommended to use non-iBind™ solutions (for example, blocking buffers, wash buffers, etc.).
	Improper application of solutions to the iBind™ Flex Wells.	Add the appropriate solutions for each well in numerical order (see page 29 for Mini Inserts, page 33 for Midi Inserts, or page 37 for Multi-Strip Inserts).

(continued)

Observation	Possible Cause	Solution
Weak or no signal	Blot was improperly placed on the iBind™ Flex Card.	• Place the membrane protein-side down in the designated Membrane Region on the iBind™ Flex Card. • Firmly roll the membrane on the iBind™ Flex Card to ensure good contact.
	Initial over-wetting of the iBind™ Flex Card.	Only use the recommended volume of 1X iBind™ Flex/iBind™ Flex FD Solution when preparing the card. Avoid adding solution to the Stack.
	Cross-contamination of solutions occurred in wells.	Do not move the iBind™ Flex device during the run.
	iBind™ Flex Card damaged.	Replace with a new card. Ensure card is free of prominent wrinkles, creases, and bends (see “iBind™ Flex Card” on page 14 for a more detailed description of acceptable card conditions). Ensure that rolling of the membrane on the card is limited to the area labeled "membrane".
	Membrane is not in proper contact with the iBind™ Flex Card.	Place the membrane on the iBind Flex Card immediately after adding the final volume of 1X iBind Flex / iBind Flex FD Solution so that it pools at the center of the membrane region on the iBind Flex Card. Use the roller provided to firmly roll the membrane on the card to ensure proper contact. When firmly rolling, the iBind Flex Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. See “Blotting roller” on page 13 for additional guidance on firm rolling.
	Device opened prior to completion of run.	The device should not be opened once the card has been placed in the device. Re-sealing of the wells on the card can result in leaks.
Oversaturated signal	Primary antibody was too concentrated.	Follow the supplier's recommended dilution or determine the optimum concentration by dot blotting.
“Spotted” membrane	Membrane was not completely wet.	Follow instructions for pre-wetting the membrane. Use an incubation dish which is small enough to allow thorough coverage of membrane to prevent drying out.
	Ink was used to label membrane.	Any labeling of the membrane with ink should be limited to the low MW region of the blot.

(continued)

Observation	Possible Cause	Solution
"Spotted" membrane	iBind™ Flex Card was damaged.	Replace with a new card. Ensure card is free of prominent wrinkles, creases, and bends (see "iBind™ Flex Card" on page 14 for a more detailed description of acceptable card conditions). Ensure that rolling of the membrane on the card is limited to the area labeled "membrane".
	Membrane was not in proper contact with the iBind™ Flex Card.	Place the membrane on the iBind™ Flex Card immediately after adding the final volume of 1X iBind™ Flex /iBind™ Flex FD Solution so that it pools at the center of the membrane region on the iBind™ Flex Card. Use the roller provided to firmly roll the membrane on the card to ensure proper contact. When firmly rolling, the iBind™ Flex Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. See "Blotting roller" on page 13 for additional guidance on firm rolling.

Troubleshooting for other western blotting factors affecting results

For a more detailed western blotting troubleshooting guide, visit **thermofisher.com/western-blotting-troubleshooting**.

Observation	Possible cause	Solution
High background	Membrane was contaminated.	Use only new, clean membranes. Wear clean gloves at all times and use forceps when handling membranes.
	Film was overexposed or became wet during exposure.	Decrease exposure time or allow signal to further decay. Prevent leakage by encasing membrane in transparency film and blotting excess substrate from edges before exposure.
	Solutions or incubation tray were contaminated.	Use clean glassware and purified water to prepare solutions. Replace or clean the tray thoroughly with a glassware-cleaning detergent. Rinse thoroughly with purified water. Wear clean gloves at all times.
Nonspecific binding	Membrane was contaminated by fingerprints or keratin proteins.	Wear clean gloves at all times and use forceps when handling membranes. Always handle membranes around the edges.
	Affinity of the primary antibody for the protein standards.	Check with protein standard manufacturer for homologies with primary antibody.

(continued)

Observation	Possible cause	Solution
Weak or no signal	Poor or incomplete transfer.	Repeat blot. After blotting, stain membrane to measure transfer efficiency. Use a positive control and/or molecular weight marker.
	Inactive primary antibody was used.	Determine activity by performing a dot-blot.
	Low affinity of primary antibody to antigen.	Obtain a higher affinity primary antibody.
	Contaminated secondary antibody solution was used.	Wear gloves at all times and keep bottles tightly capped when not in use. Use only purified water when preparing reagents.
	Protein of interest ran off the gel.	Match gel separation range to size of protein being transferred.
	Poor retention of proteins.	Match gel separation range to size of protein being transferred. Use a molecular weight marker with relevant size proteins. Larger proteins require more transfer time, smaller proteins less. Use membrane with the appropriate binding capacity.
	Sample was improperly prepared; antigenicity was weakened or destroyed.	SDS and reducing agents may interfere with some antibody/antigen affinities.
	Sample was too dilute.	Load a higher concentration or amount of protein onto the gel.
	Protein was weakly bound to membrane.	Ensure that transfer buffer contains 10–20% methanol.
	Insufficient exposure time was used.	Re-expose film for a longer period of time.
	Insufficient substrate incubation was used.	Perform each step for the specified amount of time or remove blot from substrate when signal-to-noise ratio is acceptable.
	Substrate was contaminated.	Wear gloves at all times and keep bottles tightly capped when not in use.
	Blots were too old.	Protein may have broken down over time. Use freshly prepared blots.
Oversaturated signal	Protein was overloaded.	Reduce load or dilute concentration of the sample.

(continued)

Observation	Possible cause	Solution
"Spotted" membrane	Membrane pads were dirty or contaminated.	Soak with detergent and rinse thoroughly with purified water before use. Replace pads when they become worn or discolored.
	Membrane was contaminated by fingerprints or keratin proteins.	Wear clean gloves at all times and use forceps when handling membranes. Always handle membranes around the edges.

B

Related products

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Product	Amount	Catalog No.
iBind™ Flex Western Device	1 device	SLF2000
iBind™ Flex Cards	10 cards	SLF2010
iBind™ Flex Fluorescent Detection (FD) Solution Kit	1 kit	SLF2019
iBind™ Flex Solution Kit	1 kit	SLF2020
iBind™ Flex Midi Insert Replacement	1 insert	SLF2001
iBind™ Flex Mini Insert Replacement	1 insert	SLF2002
iBind™ Flex Multi-Strip Insert Replacement	1 insert	SLF2006
Blotting Roller	1 roller	LC2100
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488	1 mg	A32731
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 555	1 mg	A32727
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647	1 mg	A32728
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 800	1 mg	A32735
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP	2 mL	31460
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	2 mL	31430
iBlot™ 3 Western Blot Transfer System	1 device	IB31001
iBlot™ 3 Transfer Stacks, midi, nitrocellulose	10 stacks	IB33001
iBlot™ 3 Transfer Stacks, midi, PVDF	10 stacks	IB34001
iBlot™ 3 Transfer Stacks, mini, nitrocellulose	10 stacks	IB33002
iBlot™ 3 Transfer Stacks, mini, PVDF	10 stacks	IB34002

C

Product specifications

iBind™ Flex Western Device specifications

Dimensions	30.0 cm (l) × 25.2 cm (w) × 8.0 cm (h)
Material	Aluminum, plastic (PC/ABS), steel, silicone, neodymium (magnets)
Operating temperature	18°C to 30°C
Temperature limit	30°C

The iBind™ Flex Western Device is impervious to alcohol, but not compatible with chlorinated hydrocarbons (for example, chloroform), aromatic hydrocarbons (for example, toluene, benzene), or acetone.

iBind™ Flex Card specifications

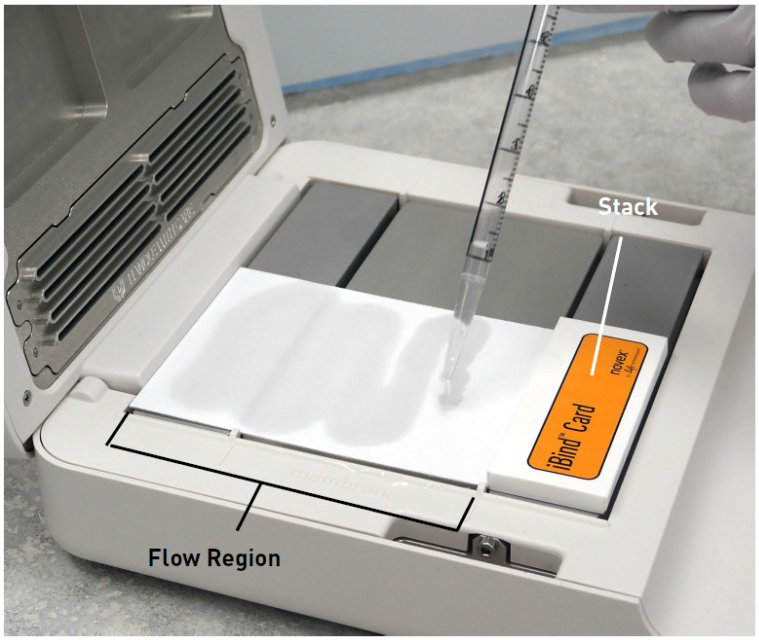
Dimensions	17.8 cm (l) × 17.8 cm (w) × 0.8 cm (stack height)
Material	Glass fiber
Operating temperature	18°C to 30°C
Temperature limit	30°C

D

Use the iBind™ Flex device with an iBind™ Card

Prepare the iBind™ Card

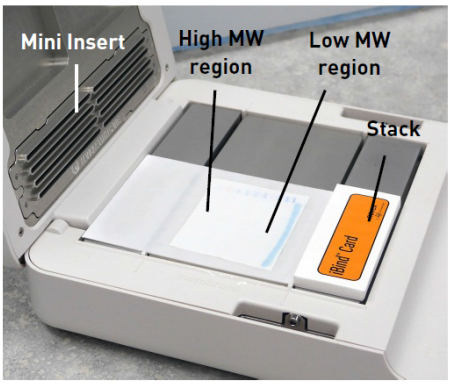
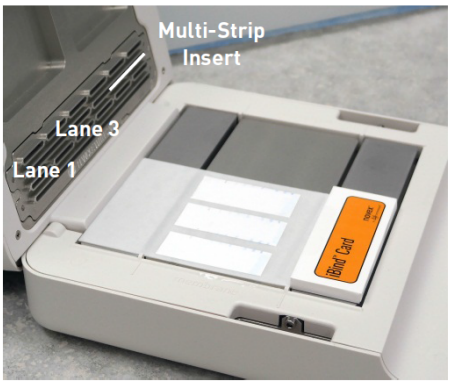
1. Open the lid of the iBind™ Flex device.
2. Verify the Mini Insert or Multi-Strip Insert is inserted in the iBind™ Flex device (depending upon the type of blot being processed).
3. Open the packaging and remove the iBind™ Card, grasping the card by the stack.
4. Inspect the condition of the iBind™ Card. If cracks, creases, or prominent wrinkles are observed, do not use the card. See “iBind™ Flex Card” on page 14 for a more detailed description of acceptable card conditions, which also apply to the iBind™ Card.
5. Place the iBind™ Card on the Stage in the position corresponding to lane 1 of the Mini Insert or lanes 1–3 of the Multi-Strip Insert.
6. Pipette 5 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution evenly across the Flow Region.



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

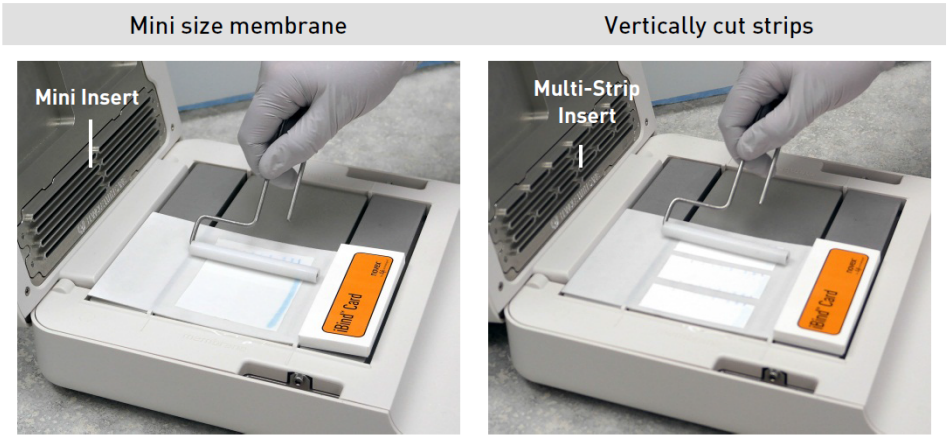
Place membrane on the iBind™ Card

1. Place the membrane(s) on top of the pooled solution with the **protein-side down**, and the low molecular weight protein region closest to the Stack. **Do not** allow the membrane to come in contact with the Stack.

Mini size membrane	Vertically cut strips
	

2. Use the Blotting Roller to firmly roll membrane on the iBind™ Card to ensure good contact and remove any air bubbles.

Note: When firmly rolling, the iBind™ Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. See Figure 3 for additional rolling details.



3. Ensure the iBind™ Card is flush against the alignment tabs, and that the membrane is within the boundaries of the membrane region. **No part of the membrane should be directly under the well insert.**

4. Lower the lid of the iBind™ Flex device and close the latch handle to lock the lid.

Add solutions to wells

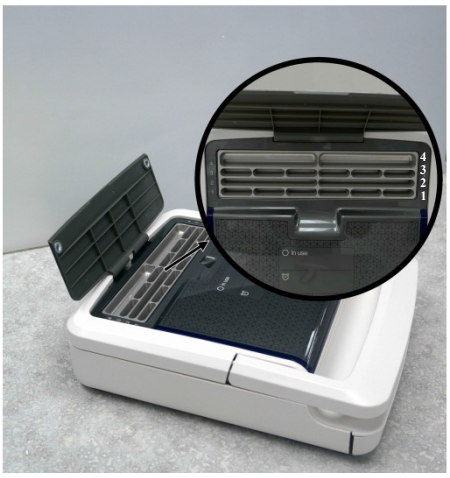
1. Open the Well Cover and add solutions sequentially to the iBind™ Flex Wells **starting with row 1** (do not exceed the total volume/well).

Row	Solution[1]	Volume/Well	
		Mini Blot	Vertically Cut Strip(s)
1	Diluted primary antibody[2,3]	2 mL	0.7 mL
2	1X iBind™ Flex/iBind™ Flex FD Solution	2 mL	2 mL
3	Diluted secondary antibody[3]	2 mL	0.7 mL
4	1X iBind™ Flex/iBind™ Flex FD Solution	6 mL	6 mL

[1] If not all lanes are being used to process vertically cut strips, add water at the given volume for each row in the unused lane(s) for wells in contact with the card. Wells that are not in contact with the card should remain empty.

[2] If processing vertically cut strips, a different antibody can be used for each lane. Antibodies can also be multiplexed for fluorescent detection (see "Multiplexing antibodies" on page 25 for more details).

[3] **Conjugated Primary Antibody Loading Procedure**: When using a conjugated primary antibody and no secondary antibody, the diluted primary antibody solution is added to well 1. Diluted secondary antibody (well 3) is replaced with 1X iBind™ Flex/iBind™ Flex FD Solution. **Alternative Conjugated Primary Antibody Loading Procedure**: For faster time to results, add diluted primary antibody to well 1, leave wells 2 and 3 empty, and add 1X iBind™ Flex/ iBind™ Flex FD Solution to well 4. This procedure will save time (approximately 60 min.) but may result in higher background.

Mini Insert	Multi-Strip Insert
	

2. Close the Well Cover and write the start time of incubation on the lid of the iBind™ Flex device.
3. Incubate for 2.5 hours or longer.

   Note: Membranes can be left in the iBind™ Flex device overnight, but perform detection as soon as possible to avoid loss of sensitivity.

4. Open the Well Cover to verify that the well(s) in row 4 are completely empty (indicating that the run is over) before releasing the latch handle and opening the lid.
5. After incubation, rinse the membrane twice in 20 mL of distilled water for 2 minutes and proceed to the preferred detection protocol.
6. Discard the iBind™ Card after use.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.