invitrogen

iBind™ Western System

USER GUIDE

For western detection of proteins on PVDF or nitrocellulose membranes

Catalog Numbers SLF1000

Publication Number MAN0008949

Revision D

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

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0008949 D (English)

Revision	Date	Description
D	11 March 2025	Manual was updated into CCMS formatting with revisions to match the iBind™ Flex Western System User Guide.
C	30 October 2014	Baseline user guide.

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

Contents

1 iBind™ Western System

Product information

Overview

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

The iBind™ Western System uses no external power source, and relies on mechanical pressure from the iBind™ Western Device on an iBind™ 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).

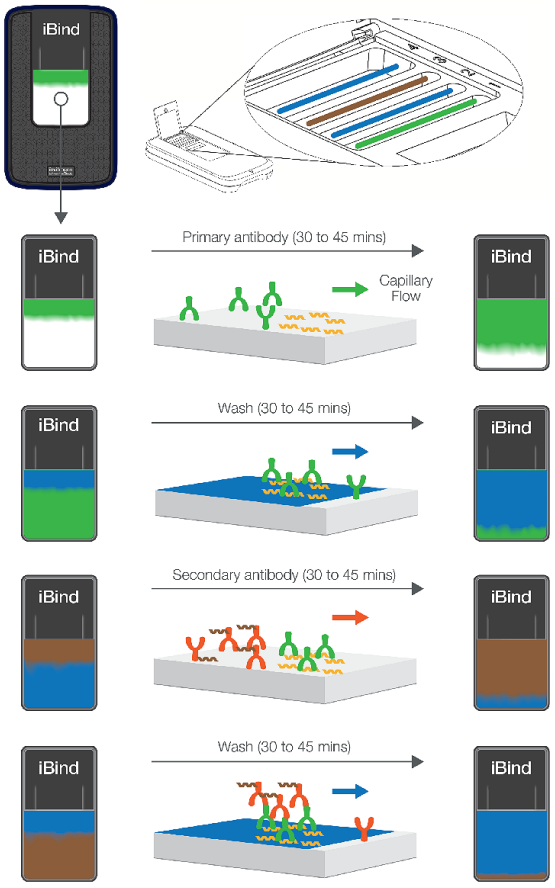


Figure 1 Sequential lateral flow technology employed by the iBind™ Western System.

Figure 2 iBind™ Western System

System components

The iBind™ Western System consists of:

- iBind™ Western Device
- iBind™ Cards
- iBind™ Solution Kit
- iBind™ Fluorescent Detection (FD) Solution Kit

Contents

The components included with the iBind™ Western Device (Cat. No. SLF1000) are listed below.

Components	Quantity
iBind™ Western Device	1 unit
iBind™ Blotting Roller	1 roller
iBind™ Window Cover	1 unit

Required materials not supplied with the device

iBind™ Cards

The iBind™ Card is a unique matrix optimized for homogenous flow of immunodetection reagents along its length. The iBind™ Cards are sold separately (see “Related products” on page 32 for ordering details).

The components included with the iBind™ Cards (Cat. No. SLF1010) are listed below.

Product	Quantity	Storage
iBind™ Card	10 cards	Room temperature

iBind™ Solution Kit

The iBind™ Solution Kit is used for preparing blocking, dilution, and washing buffers for the iBind™ western detection protocol using chemiluminescent or colorimetric detection. The iBind™ Solution Kit is sold separately (see “Related products” on page 32 for ordering details).

The components included with the iBind™ Solution Kit (Cat. No. SLF1020) are listed below.

Product	Quantity	Storage
iBind™ 5X Buffer	60 mL	4°C
iBind™ 100X Additive	2 x 1.5 mL	4°C

iBind™ Fluorescent Detection (FD) Solution Kit

The iBind™ Fluorescent Detection (FD) Solution Kit is used for preparing blocking, dilution, and washing buffers for the iBind™ western detection protocol in conjunction with fluorophore-conjugated secondary antibodies (for example, Alexa Fluor™ Plus Secondary Antibodies). The iBind™ Fluorescent Detection (FD) Solution Kit is sold separately (see “Related products” on page 32 for ordering details).

The components included with the iBind™ Fluorescent Detection (FD) Solution Kit (Cat. No. SLF1019) are listed below.

Product	Quantity	Storage
iBind™ FD 5X Buffer	60 mL	4°C
iBind™ 100X Additive	2 x 1.5 mL	4°C
iBind™ FD 10% SDS	100 µL	Room temperature

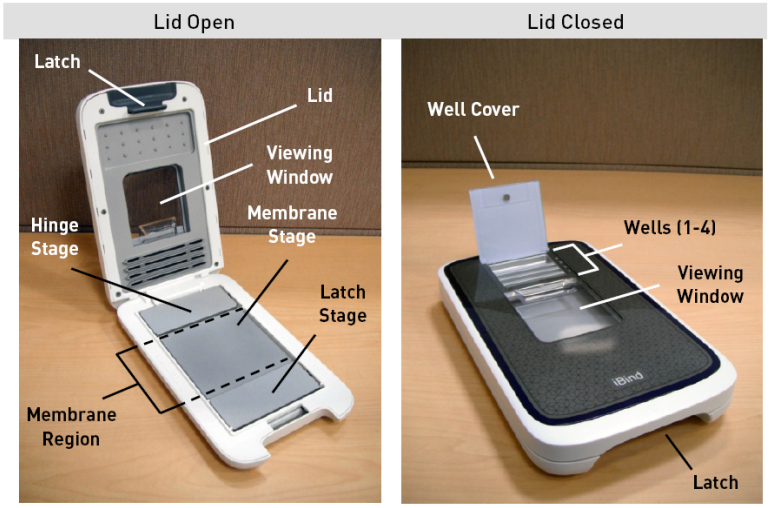
Description of parts

iBind™ Western Device

The iBind™ Western Device consists of a metallic surface made up of three stages and a lid with four wells for loading blocking solution, antibodies, and wash solutions.

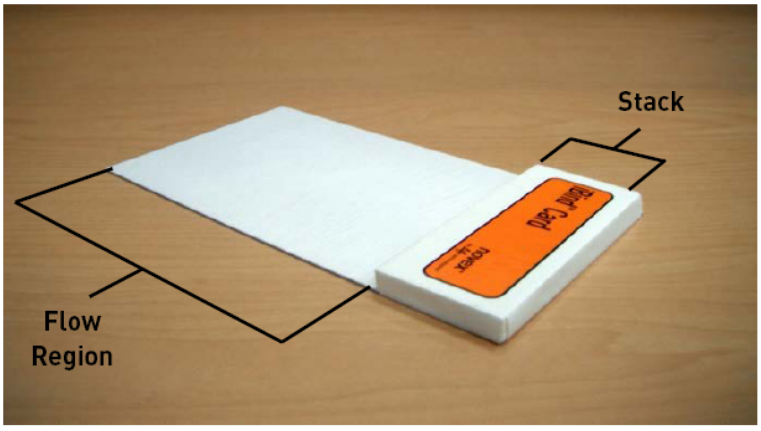
The Hinge Stage and Latch Stage are spring plates designed to apply specific amounts of pressure on an iBind™ Card placed on the 3 stages, when the lid of the device is locked.

The pressure on the iBind™ 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.



iBind™ Card

The iBind™ Card consists of a Flow Region and a Stack. The card is a unique matrix optimized for homogenous flow of immunodetection reagents along the Flow Region.



IMPORTANT!

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

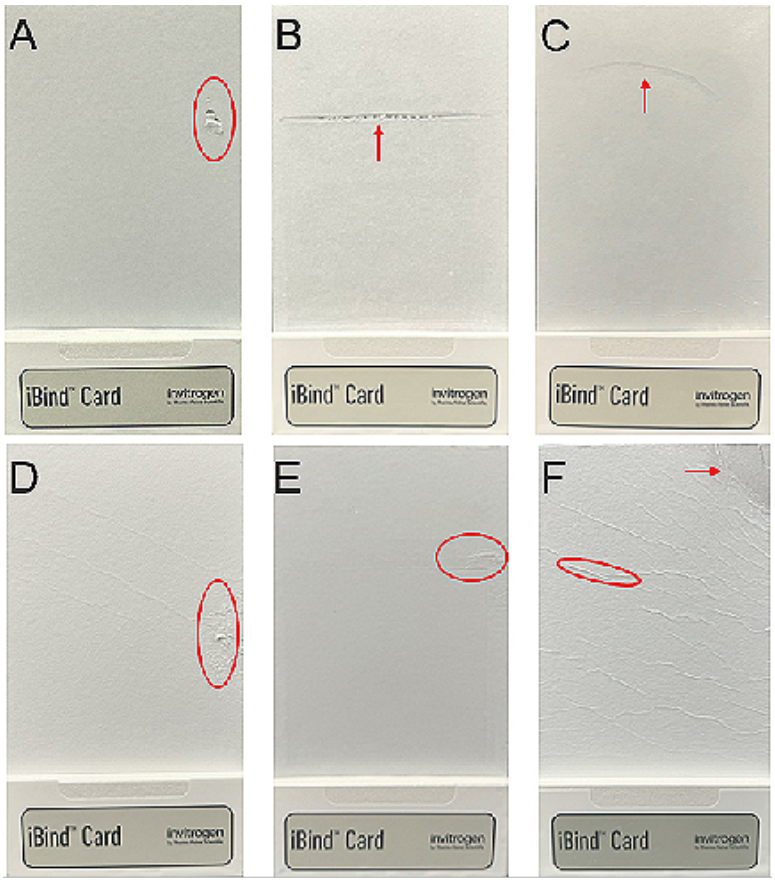


Figure 3 Examples of common iBind™ Card mishandling. iBind™ Cards should be handled by the stack to prevent damage prior to processing. Damage to the card can be caused by lab tools such as tweezers, rollers, and pipettes. Damage may also happen from mishandling of the card by the user. Using damaged 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.

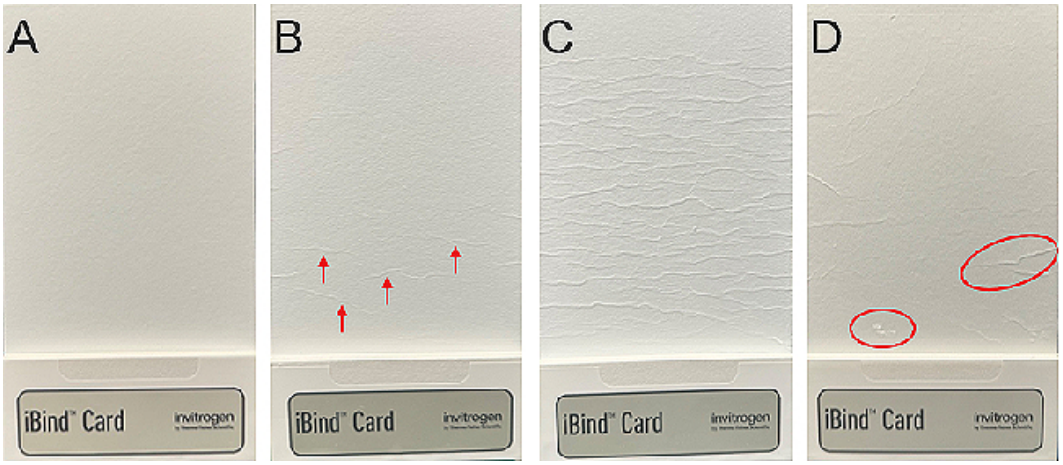
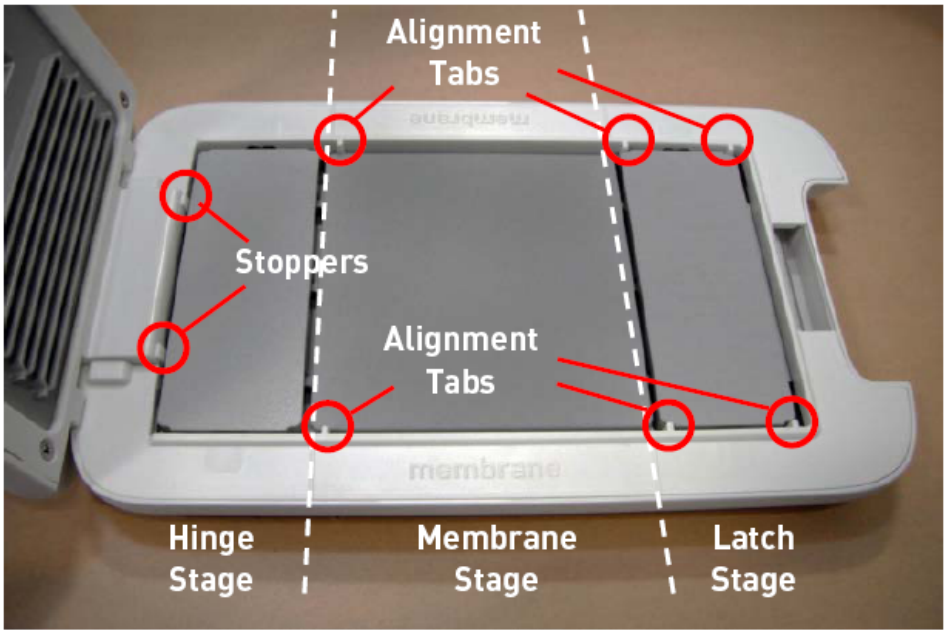


Figure 4 Acceptable and unacceptable conditions of iBind™ Cards. iBind™ Cards should be inspected for cracks, creases, and prominent wrinkles before using. Cards in unacceptable condition should not be used. Cards with few or no wrinkles are acceptable to use. Cards with many wrinkles, prominent wrinkles, 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 and large fiber clumps.

The iBind™ Card is placed on the iBind™ device so that it is aligned with the stoppers and alignment tabs.

Note: Ensure the lid is completely open to expose the stoppers near the hinge.



iBind™ window cover

The iBind™ Window Cover is an opaque rubber cover placed over the viewing window when using light sensitive reagents to perform western detection.

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™ Card. **Firm rolling is required to ensure optimal results.**

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

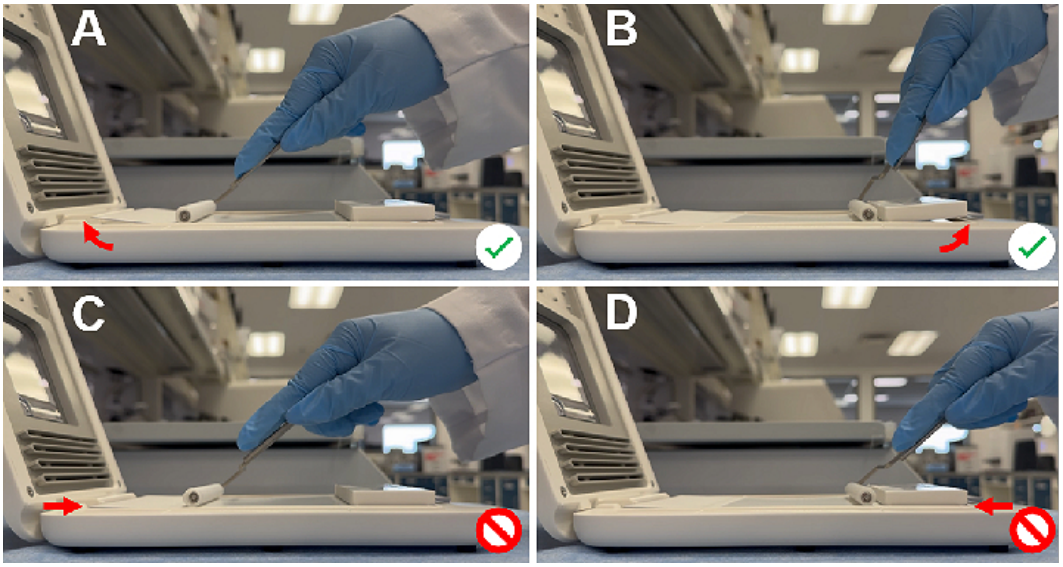


Figure 5 Firm rolling guidance. Firmly rolling the membrane on the iBind™ Card ensures good contact and removes any air bubbles that affect immunoprocessing of the blot. After placing the membrane protein-side down on the iBind™ Card, firmly roll the membrane on the card with a Blotting Roller to remove any air bubbles. (A and B) When firmly rolling the membrane, the iBind™ 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™ Card. Dead bands and fuzziness can occur if there is poor contact between the membrane and card.

2 Methods

Procedural overview

General guidelines

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

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

IMPORTANT! Handle iBind™ Cards with care as bent, creased, or prominently wrinkled cards can result in poor immunodetection. See Figure 4 for a more detailed description of acceptable card conditions).

- Wear the proper protective equipment (gloves, laboratory coat, eye protection) when performing experiments.
- If you mark your membrane with ink, mark the membrane near the low molecular weight region.
- 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 5 for additional details.
- Do not move the iBind™ device or open the lid until Well 4 is completely empty (2.5 hours or longer).

  Note: The membrane can be left in the iBind™ device overnight if desired.
- The 1X iBind™ Solution is used for chemiluminescent detection, while the 1X iBind™ FD Solution is used for fluorescent detection.
- For best results, use a 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).
- The iBind™ device is compatible with conjugated primary antibodies where no secondary antibody is needed. See step 5 for additional details.
- The iBind™ device can be used to reprobe stripped blots. It is not recommended to strip the blot using the iBind™ device.
- The iBind™ device is compatible with multiplexing. See “Multiplexing antibodies” on page 17 for more details.

Chemiluminescent detection procedure

Experimental overview

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

Step	Action	Page
1	Prepare 1X iBind™ Solution	"Prepare 1X iBind™ Solution" on page 14
2	Prepare membrane(s)	"Prepare membrane(s)" on page 14
3	Prepare diluted antibody solutions	"Prepare antibody solutions" on page 15
4	Prepare iBind™ Card	"Using the iBind™ device" on page 18
5	Add solutions to iBind™ Wells and incubate 2–3 hours	"Perform antibody binding" on page 20
6	Perform detection	

Prepare 1X iBind™ Solution

The 1X iBind™ Solution is used for blocking, diluting antibodies, washing, and wetting the iBind™ Card. Prepare 30 mL of 1X iBind™ Solution for each run as follows:

Reagent	Volume
iBind™ 5X Buffer	6 mL
iBind™ 100X Additive	300 µL
Distilled water	23.7 mL
Total	**30 mL**

Prepare membrane(s)

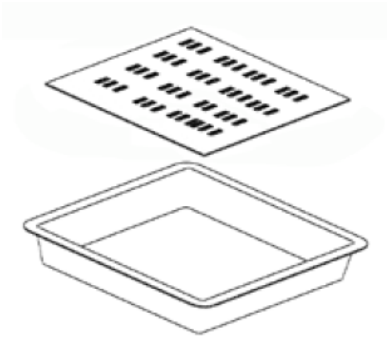
It is recommended to proceed with blocking and iBind™ processing intermediately after transfer. If storage of membranes is required prior to processing, store membranes in distilled water or dry.

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

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

- Trim the membrane to 9 cm x 9 cm.
- Pre-activate PVDF membranes in 100% methanol, and rinse in distilled water.

- Immerse the blotted membrane (**protein-side up**) in 5 mL of 1X iBind™ Solution. Incubate for 2–10 minutes with or without shaking.



Prepare antibody solutions

Dilute primary and secondary antibodies in 2 mL each of 1X iBind™ Solution according to recommendations in the table below.

Prepare primary antibody solution	
Component	**Volume**
1X iBind™ Solution	2 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	**Volume**
1X iBind™ Solution	2 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 "Prepare iBind™ Card" on page 18 to begin preparing the iBind™ Card.

Fluorescent detection procedure

Experimental overview

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

Step	Action	Page
1	Prepare 1X iBind™ FD Solution	"Prepare 1X iBind™ FD solution" on page 16
2	Prepare membrane	"Prepare membrane(s)" on page 17
3	Prepare diluted antibody solutions	"Prepare antibody solutions" on page 18
4	Prepare iBind™ Card	"Prepare iBind™ Card" on page 18
5	Add solutions to iBind™ Wells and incubate 2–3 hours	"Perform antibody binding" on page 20
6	Perform detection	

Prepare 1X iBind™ FD solution

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

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

Prepare 30 mL of 1X iBind™ FD Solution for each run as follows:

Reagent	Volume	
	Standard	Optional
iBind™ FD 5X Buffer	6 mL	1.5 mL
iBind™ 100X Additive	75 µL	300 µL
Distilled Water	23.9 mL	28.2 mL
Total	30 mL	30 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™ FD Solution. Use of other blockers may interfere with iBind™ processing.

- Trim the membrane to 9 cm x 9 cm.
- Pre-activate PVDF membranes in 100% methanol, and rinse in distilled water.
- Immerse the blotted membrane (**protein-side up**) in 5 mL of 1X iBind™ FD Solution. Incubate for 2–10 minutes, with or without shaking.

Multiplexing antibodies

Antibodies can be multiplexed to perform detection when using the iBind™ device.

- **Primary antibodies**: Prepare appropriate primary antibodies together in a single tube of 1X iBind™ FD Solution. All primary antibodies should be diluted according to the recommendations outlined in the table below.
- **Secondary antibodies**: Prepare appropriate secondary antibodies together in a single tube of 1X iBind™ 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 2 mL each of 1X iBind™ FD Solution according to recommendations in the table below.

Prepare primary antibody solution	
Component	**Volume**
1X iBind™ FD Solution	2 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	**Volume**
1X iBind™ FD Solution	2 mL
iBind™ FD 10% SDS[2]	10 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.

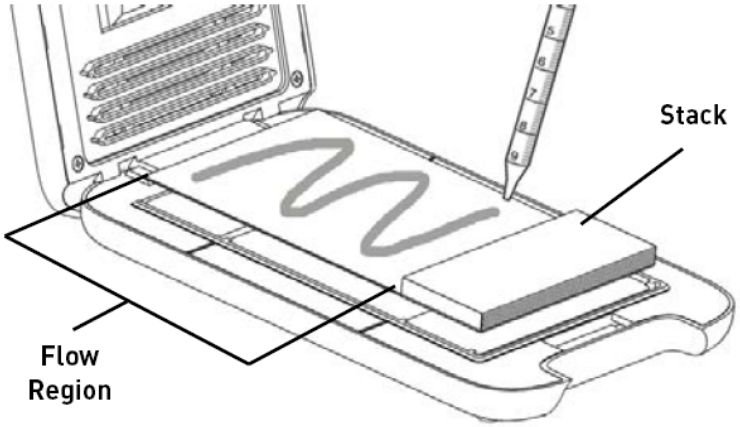
[2] SDS is added to a final concentration of 0.05% to reduce background signal, particularly when using PVDF membranes or fluorophore-conjugated secondary antibodies.

Using the iBind™ device

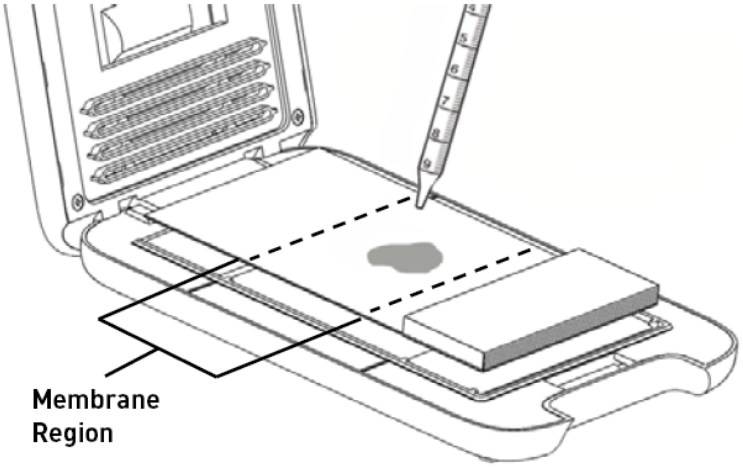
Prepare iBind™ Card

1. Open the lid of the iBind™ device.
2. Open the packaging and remove the iBind™ Card by grasping the card by the stack.
3. Inspect the condition of the iBind™ Card. If cracks, creases, or prominent wrinkles are observed, do not use the card. See "iBind™ Card" on page 8 for a more detailed description of acceptable card conditions.
4. Place the iBind™ Card on the Stage and align it with the stoppers (see "iBind™ Card" on page 8 for details).

5. Pipette 5 mL of 1X iBind™/ iBind™ FD Solution evenly across the Flow Region.

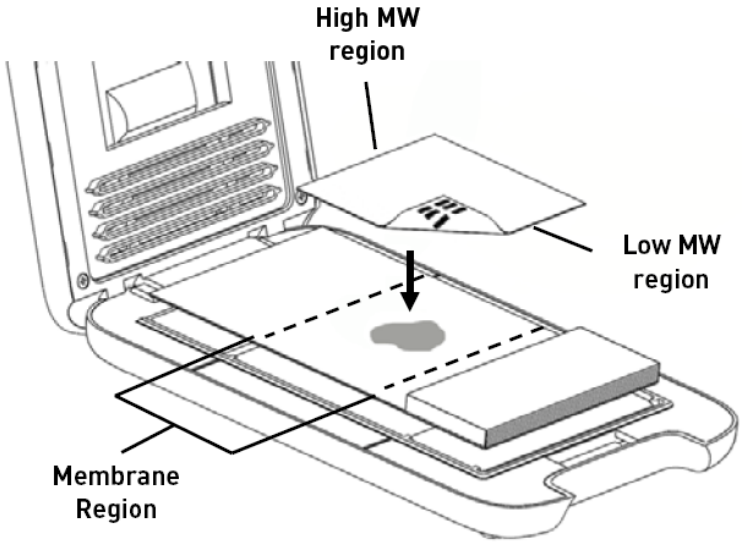


6. Pipette 1 mL of 1X iBind™/iBind™ FD Solution so that it pools at the center of the membrane region on the iBind™ Card.



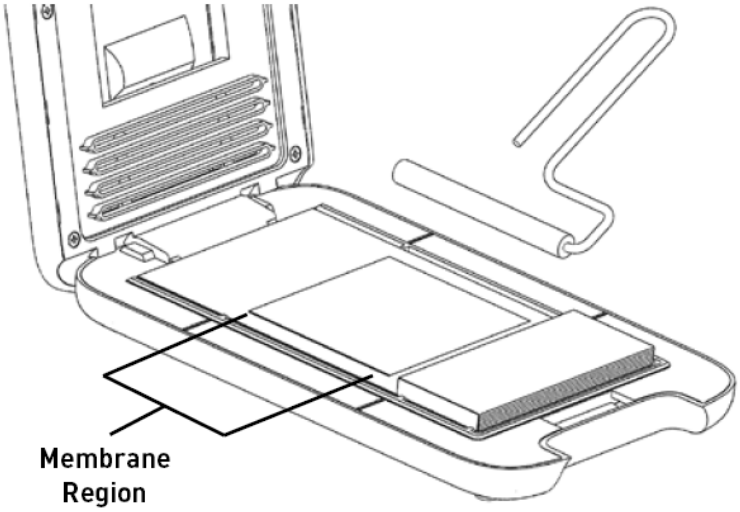
Perform antibody binding

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.



2. Use the blotting roller to remove any air bubbles between the membrane and the iBind™ Card.

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 5 for additional detail.



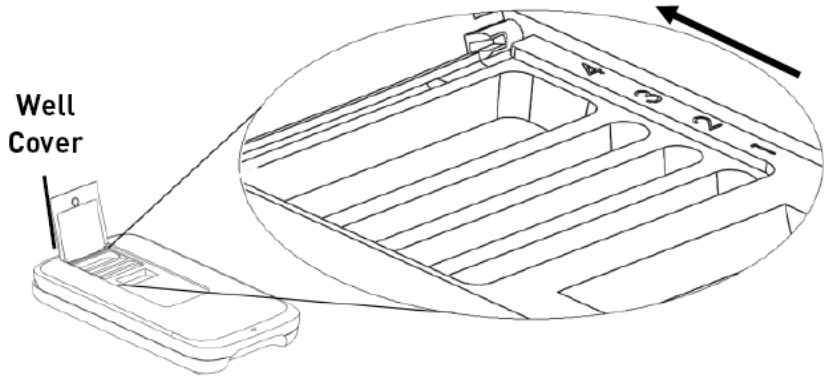
3. Ensure the iBind™ Card is aligned against the stoppers and the membrane is within the boundaries of the membrane region. **No part of the membrane should be directly under the wells.**

4. Close the device lid. A clicking sound indicates the lid is properly locked.

5. Open the Well Cover and add solutions sequentially to the iBind™ wells starting with Well 1.

Well	Solution
1	2 mL diluted primary antibody[1]
2	2 mL of 1X iBind™/iBind™ FD Solution
3	2 mL diluted secondary antibody
4	6 mL of 1X iBind™/iBind™ FD Solution

[1] **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™/iBind™ 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™/iBind™ FD Solution to Well 4. This procedure will save time (approximately 60 minutes) but may result in higher background.



6. Place the iBind™ Window Cover over the viewing window.

7. Close the Well Cover and incubate for ≥2.5 hours.

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

8. Open the Well Cover to verify that Well 4 is completely empty (indicating that the run is over) before opening the lid.

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

Note: Blots processed with iBind™ device can be stripped and reprobed. After the stripping protocol, the blot can be reprobed with a new iBind™ 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™ device.

Maintenance

General guidelines

- Rinse the iBind™ device under running water after each use and allow the device to dry before additional usage.
- To maximize the life of the springs in iBind™ 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™ Card condition

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

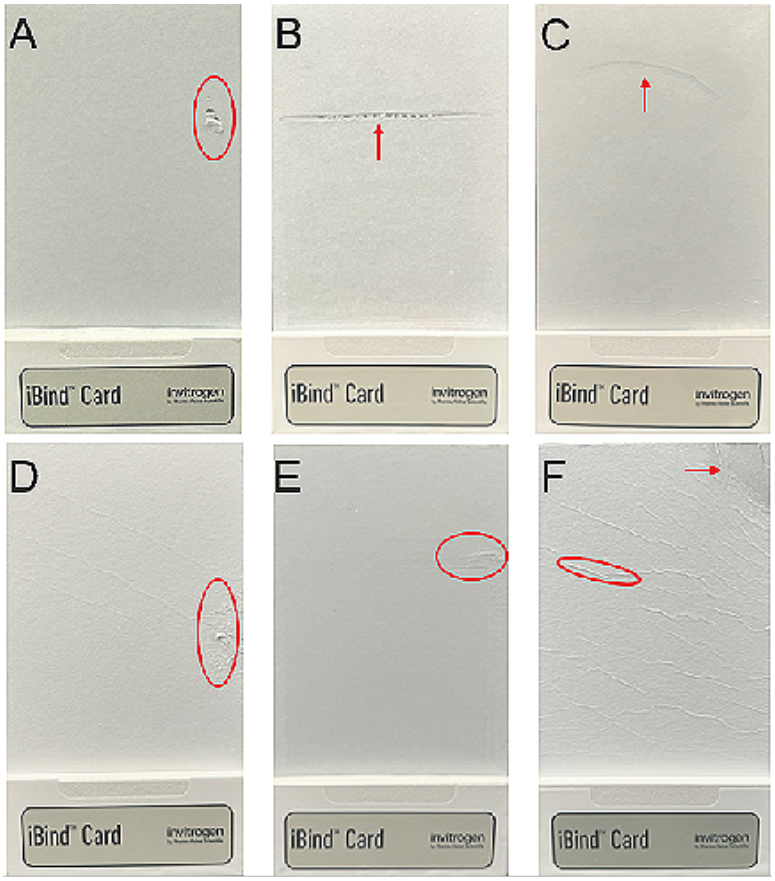


Figure 6 Examples of common iBind™ Card mishandling. iBind™ Cards should be handled by the stack to prevent damage prior to processing. Damage to the card can be caused by lab tools such as tweezers, rollers, and pipettes. Damage may also happen from mishandling of the card by the user. Using damaged 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.

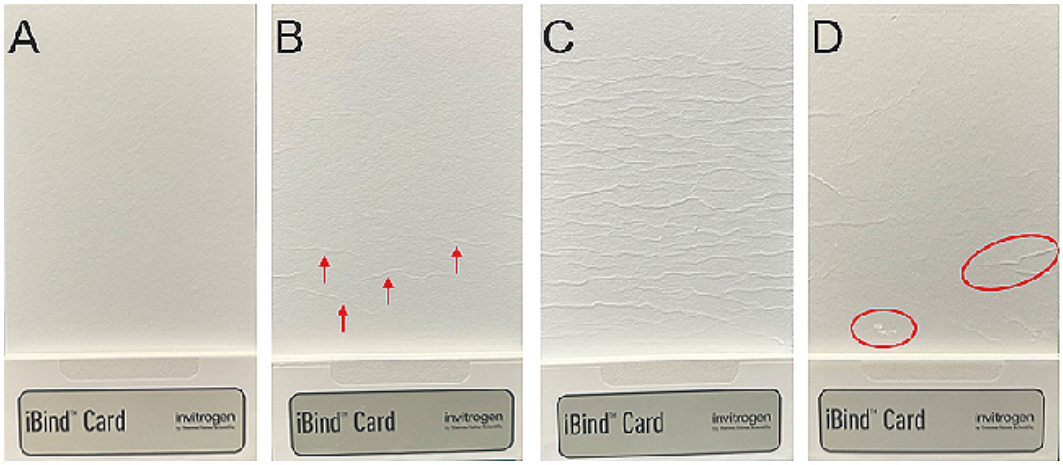


Figure 7 Acceptable and unacceptable conditions of iBind™ Cards. iBind™ Cards should be inspected for cracks, creases, and prominent wrinkles before using. Cards in unacceptable condition should not be used. Cards with few or no wrinkles are acceptable to use. Cards with many wrinkles, prominent wrinkles, 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 and large fiber clumps.

Firm rolling

Firm rolling is needed to ensure optimal results. When firmly rolling, the iBind™ 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™ Card.

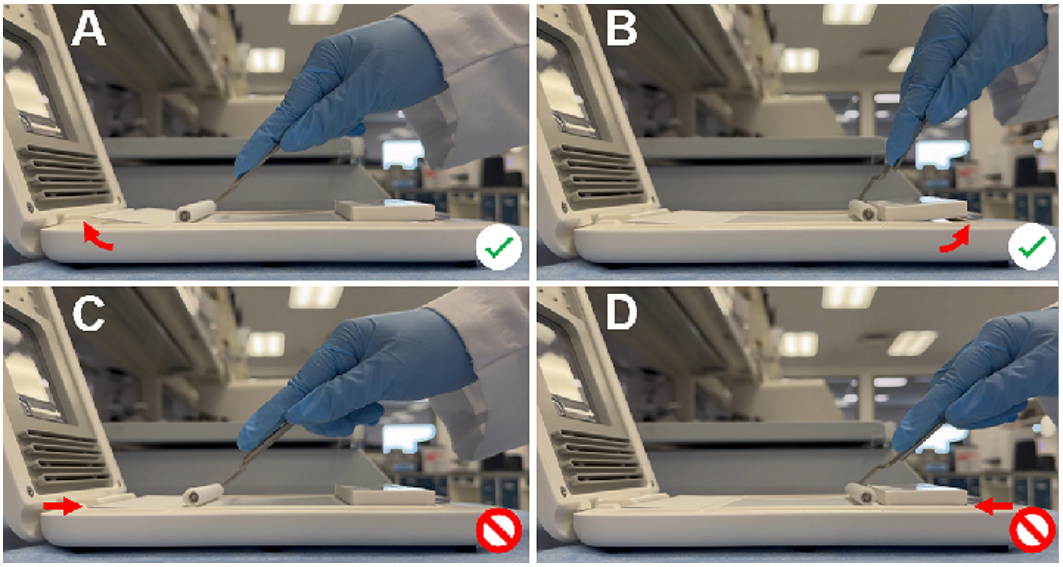


Figure 8 Firm rolling guidance. Firmly rolling the membrane on the iBind™ Card ensures good contact and removes any air bubbles that affect immunoprocessing of the blot. After placing the membrane protein-side down on the iBind™ Card, firmly roll the membrane on the card with a Blotting Roller to remove any air bubbles. (A and B) When firmly rolling the membrane, the iBind™ 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™ Card. Dead bands and fuzziness can occur if there is poor contact between the membrane and card.

Troubleshooting the iBind™ device

Observation	Possible Cause	Solution
Run times greater than 3 hours	iBind™ Card was damaged.	Replace with a new card. Ensure card is free of prominent wrinkles, creases, and bends (see "iBind™ Card" on page 8 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™ Card.	Only use the recommended volume of 1X iBind™/iBind™ FD Solution when preparing the card. Avoid adding solution to the Stack.
	Improper preparation of iBind™/iBind™ FD Solution.	Prepare 1X iBind™/iBind™ FD Solutions as directed ("Prepare 1X iBind™ Solution" on page 14 or "Prepare 1X iBind™ FD solution" on page 16). It is not recommended to use non-iBind™ solutions (for example, blocking buffers, wash buffers, etc.).
	Improper blocking buffer was used.	The iBind™/iBind™ FD Solution is used as a combined blocking, washing, and antibody diluent solution. The iBind™ and iBind™ 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 14 (chemiluminescent) or "Prepare membrane(s)" on page 17 (fluorescent)]. 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™/iBind™ FD Solution.	Prepare 1X iBind™/iBind™ FD Solutions as directed ("Prepare 1X iBind™ Solution" on page 14 or "Prepare 1X iBind™ FD solution" on page 16). It is not recommended to use non-iBind™ solutions (for example, blocking buffers, wash buffers, etc.).
	Solutions were improperly applied to iBind™ wells.	Add the appropriate solutions for each well in numerical order ("Perform antibody binding" on page 20).

(continued)

Observation	Possible Cause	Solution
High background	Blot was improperly placed on the iBind™ Card.	Ensure the protein side of the blot is in contact with the iBind™ Card.
	Card stack was wet prior to run.	Ensure that 5 mL of 1X iBind™/iBind™ 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™ Solution" on page 14 or "Prepare 1X iBind™ FD solution" on page 16).
	Improper preparation of iBind™/iBind™ FD Solution.	Prepare 1X iBind™/iBind™ FD Solutions as directed ("Prepare 1X iBind™ Solution" on page 14 or "Prepare 1X iBind™ FD solution" on page 16).
Weak or no signal	Membrane was not completely wet.	Follow instructions for pre-wetting the membrane [see "Prepare membrane(s)" on page 14 (chemiluminescent) or "Prepare membrane(s)" on page 17 (fluorescent)]. 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™/iBind™ FD Solution.	Prepare 1X iBind™/iBind™ FD Solutions as directed ("Prepare 1X iBind™ Solution" on page 14 or "Prepare 1X iBind™ FD solution" on page 16). It is not recommended to use non-iBind™ solutions (for example, blocking buffers, wash buffers, etc.).
	Improper application of solutions to the iBind™ wells.	Add the appropriate solutions for each well in numerical order ("Perform antibody binding" on page 20).
	Blot was improperly placed on the iBind™ Card.	Ensure the protein side of the blot is in contact with the iBind™ Card and is placed in the region labeled "membrane".

(continued)

Observation	Possible Cause	Solution
Weak or no signal	Initial over-wetting of the iBind™ Card.	Only use the recommended volume of 1X iBind™/iBind™ FD Solution when preparing the card. Avoid adding solution to the Stack.
	Cross-contamination of solutions occurred in wells.	Do not move the iBind™ device during the run.
	iBind™ Card was damaged.	Replace with a new card. Ensure card is free of prominent wrinkles, creases, and bends (see “iBind™ Card” on page 8 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™ Card.	Place the membrane on the iBind Card immediately after adding a 1 mL pool of 1X iBind™/iBind™ FD Solution. Use the roller provided to firmly roll the membrane on the card to ensure proper contact. When firmly rolling, the iBind™ Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. See “Blotting roller” on page 11 for additional guidance on firm rolling.
	Device was opened prior to completion of the 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.
	iBind™ Card was damaged.	Replace with a new card. Ensure card is free of prominent wrinkles, creases, and bends (see “iBind™ Card” on page 8 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™ Card.	Place the membrane on the iBind Card immediately after adding a 1 mL pool of 1X iBind™/ iBind™ FD Solution. Use the roller provided to firmly roll the membrane on the card to ensure proper contact. When firmly rolling, theiBind™ Card will slightly dip in the membrane area, causing the top and bottom of the card to lift. See “Blotting roller” on page 11 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.
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.

(continued)

Observation	Possible cause	Solution
Weak or no signal	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.
“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™ Western Device	1 device	SLF1000
iBind™ Cards	10 cards	SLF1010
iBind™ Fluorescent Detection (FD) Solution Kit	1 kit	SLF1019
iBind™ Solution Kit	1 kit	SLF1020
iBind™ Window Cover	1 unit	SLF1001
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
SuperSignal™ West Atto Ultimate Sensitivity Substrate	200 mL	A38556
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	200 mL	34577
SuperSignal™ West Dura Extended Duration Substrate	200 mL	34076
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™ Western Device specifications

Dimensions	24.2 cm (l) x 14.6 cm (w) x 3.5 cm (h)
Material	Aluminum, plastic (PC/ABS), steel, silicone, neodymium (magnets)
Operating temperature	18°C to 30°C
Temperature limit	30°C

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

iBind™ Card specifications

Dimensions	17.8 cm (l) x 10 cm (w) x 0.8 cm (stack height)
Material	Glass fiber
Operating temperature	18°C to 30°C
Temperature limit	30°C

D

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

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  - 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**.