USER GUIDE

invitrogen

iBind™ Flex Western System

Online Specials

For western detection of proteins on PVDF or nitrocellulose membranes

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Product Information

iBind™ Flex Western Device

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 Device 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.

System components

The iBind™ Flex Western System consists of:

- 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 iBind™ Flex Western Device.

iBind™ Flex Cards

The iBind™ Flex Card is a unique matrix optimized for homogenous flow of immunodetection reagents along its length (see page 10 for details).

The iBind™ Flex Cards are **single use**, and sold separately (see page 34 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 Fluorescent Detection (FD) Solution Kit is used for preparing blocking, dilution, and washing buffers for the iBind™ Flex western detection protocol in conjunction with Alexa Fluor® or IRDye® conjugated secondary antibodies. The iBind™ Flex Fluorescent Detection (FD) Solution Kit is sold separately (see page 34 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.

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

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 chromogentic substrates, and alkaline phosphatase (AP) or horseradish peroxidase (HRP) conjugated secondary antibodies. The iBind™ Flex Solution Kit is sold separately (see page 34 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.

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

Description of Parts

iBind™ Flex Western Device

The iBind™ Flex Western Device is an automated device that utilizes sequential lateral flow (SLF) to automatcally 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 four rows of wells for loading blocking solution, antibodies, and wash solutions.There are three different well inserts (for processing one midi blot, up to two mini blots, or up to six vertically cut strip blots.

Lid and Drawer Closed

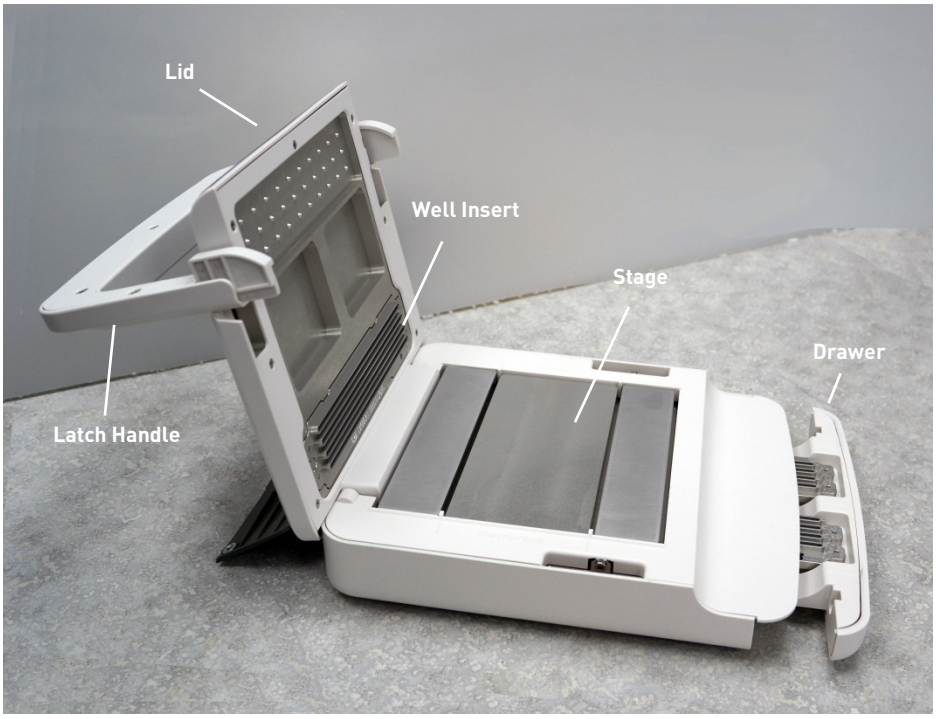
iBind™ Flex Western Device lid

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.

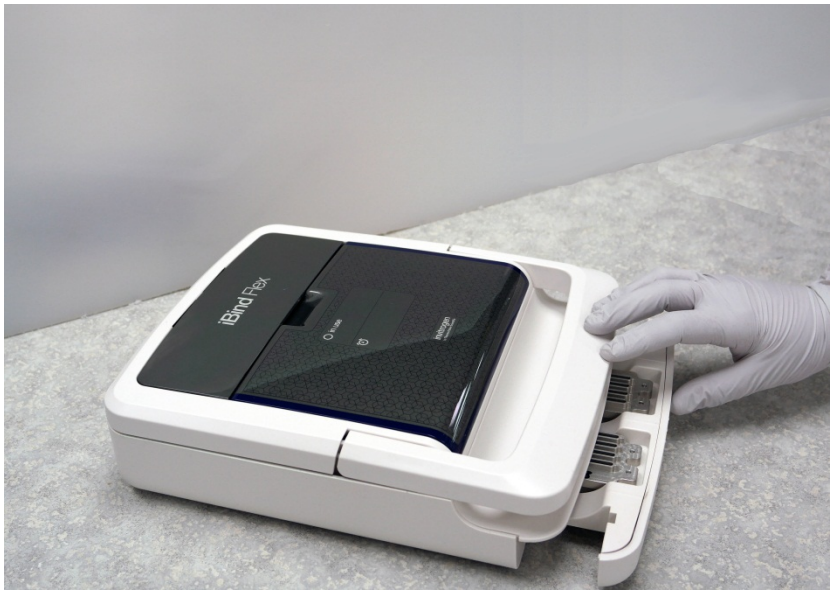
Lid and Drawer Open



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

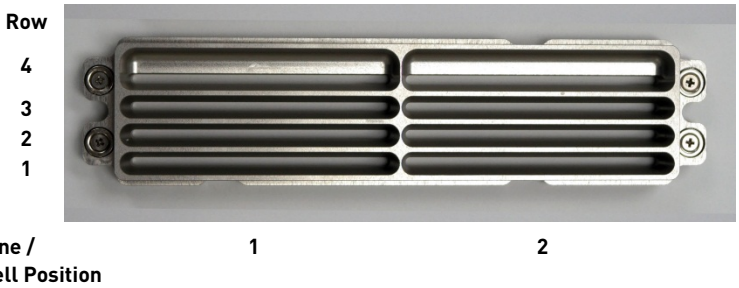
iBind™ Flex Midi Insert

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 two mini sized membranes.

Each row of the Mini Insert consists of two wells.

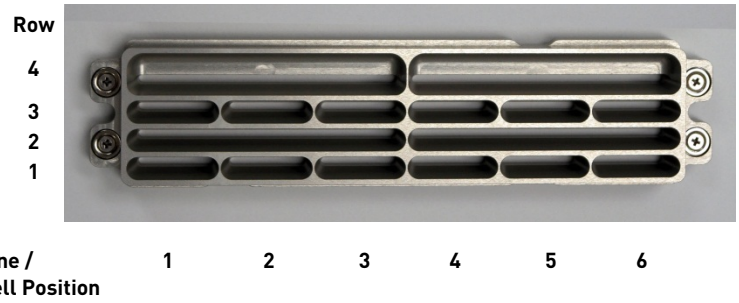


iBind™ Flex Multi-Strip Insert

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

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

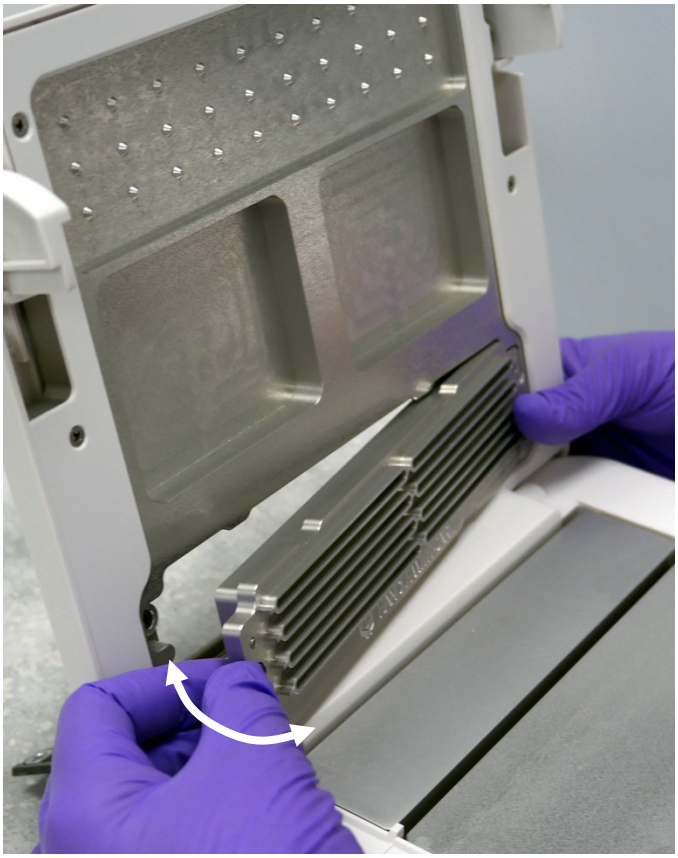
Refer to page 12 for additional details on using the iBind™ Flex Multi-Strip Insert.



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 Western Device in the correct orientation.

To install an insert:	To remove an insert:
1. Open the lid and well cover of the iBind™ Flex Western Device.	1. Open the lid and well cover of the iBind™ Flex Western Device.
2. Push the insert from the back of the lid, and slide it out of the slot.	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). The Blotting Roller is used to remove any air bubbles between the membrane and the iBind™ Flex Card.

iBind™ Flex Card

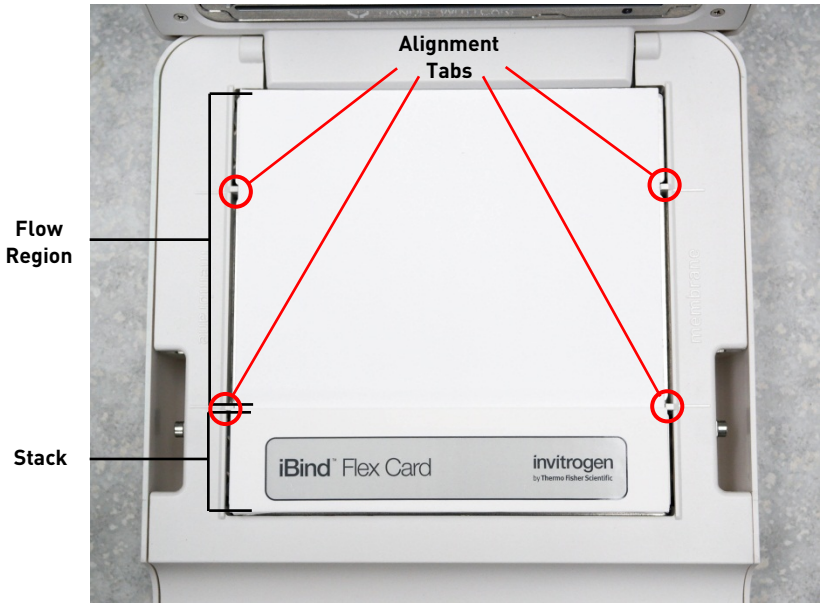
The iBind™ Flex Card is a unique glass fiber matrix optimized for homogenous flow of immunodetection reagents.

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:

- **Do not bend the iBind™ Flex Card**.
- **iBind™ Flex Cards are single use only. Discard card after use.**

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.

Methods

Procedural Overview

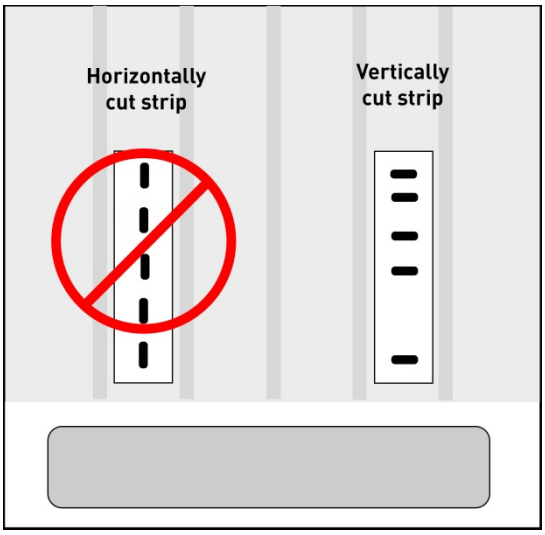
General guidelines

- 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 Western Device when not in use.
- If you mark your membrane(s) with ink, mark the membrane(s) near the low molecular weight region.
- When performing detection using iBind™ Flex Multi-Strip or Mini Inserts, a different primary antibody can be used in conjunction with the appropriate secondary antibody for each lane.
- When performing fluorescent detection using iBind™ Flex Midi, Mini, or Multi-Strip Inserts, more than one antibody can be multiplexed within a
- **Caution:** Exercise care when closing the lid of the iBind™ Flex Western Device to avoid catching fingers.
- **Important**: No part of the membrane(s) should be directly under the wells when the lid is closed.
- Do not move the iBind™ Flex Western 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 Western Device overnight if desired.
- 1X iBind™ Flex Solution is used for HRP and AP detection, while 1X iBind™ Flex FD Solution is used for fluorescent detection.

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 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

HRP and AP Detection Procedure

Experimental overview

Use the following protocol when using the iBind™ Flex Western System with HRP or AP detection protocols.

Step	Action	Page
1	Prepare 1X iBind™ Flex Solution	13
2	Prepare membrane	13
3	Prepare diluted antibody solutions	14
4	Perform antibody binding using: • Midi Insert • Mini Insert • Multi-Strip Insert	 17 20 23
5	Perform detection	26–27

Prepare 1X iBind™ Flex Solution

1X iBind™ Flex Solution is used for blocking, diluting antibodies, washing, and wetting the iBind™ Flex Card. Prepare 50 mL of 1X iBind™ Flex Solution for each run as follows:

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

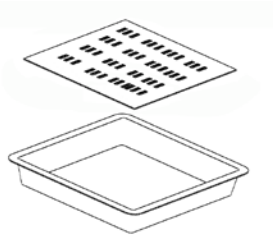
Prepare membrane

Store membranes in 1X iBind™ Flex Solution, in distilled water, or dry if they are not used immediately after transfer.

Membranes should only be blocked with 1X iBind™ Flex Solution.

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 mL for each mini sized membrane or for vertically cut strips, or 20 mL for midi sized membranes.



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).

Prepare primary antibody solution

Dilute primary antibodies with 1X iBind™ Flex Solution according to the enzyme system being used for detection.

Component	Midi Blot	Mini Blot	Vertically Cut Strip
1X iBind™ Flex Solution	4 mL	2 mL	0.7 mL
1° Antibody	Dilute antibody to manufacturer's recommended dilution.		

Prepare secondary antibody solution

Dilute primary antibodies with 1X iBind™ Flex Solution according to the enzyme system being used for detection.

Component	Midi Blot	Mini Blot	Vertically Cut Strip
1X iBind™ Flex Solution	4 mL	2 mL	0.7 mL
2° Antibody	Prepare antibody at 5X the manufacturer's recommended dilution (e.g. use 1:1000 dilution if 1:5000 dilution is recommended)		

Fluorescent Detection Procedure

Experimental overview

Use the following protocol when using the iBind™ Flex Western System in conjunction with the LI-COR® Odyssey® Imaging System.

Step	Action	Page
1	Prepare 1X iBind™ Flex FD Solution	15
2	Prepare membrane(s)	15
3	Prepare diluted antibody solutions	16
4	Perform antibody binding using: • Midi Insert • Mini Insert • Multi-Strip Insert	 17 20 23
5	Perform detection	26–27

Prepare 1X iBind™ Flex FD Solution

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 50 mL of 1X iBind™ Flex FD Solution for each run as follows:

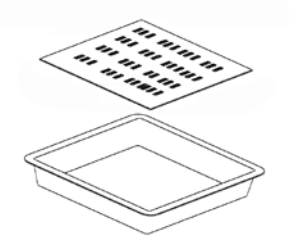
Reagent	Volume	
	Standard	Optional
iBind™ Flex FD 5X Buffer	10 mL	2.5 mL
iBind™ Flex 100X Additive	125 µL	500 µL
Distilled Water	39.9 mL	47 mL
Total	50 mL	50 mL

Prepare membrane(s)

Store membranes in 1X iBind™ Flex FD Solution, in distilled water, or dry if they are not used immediately after transfer.

Membranes should only be blocked with 1X iBind™ Flex FD Solution. Before performing the antibody binding, prepare the membrane(s) 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 FD Solution. Use 10 mL for each mini sized membrane or for vertically cut strips, or 20 mL for midi sized membranes.



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).

Multiplexing antibodies

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

Prepare primary antibodies so that the final concentration of each antibody is at the recommended dilution when combined.

Use the appropriate fluorescent secondary antibodies for multiplexed primary antibodies in the corresponding lane (well 3). Prepare secondary antibodies so that the final concentration of each antibody is at the recommended dilution when combined.

Prepare primary antibody solution

Dilute antibodies with 1X iBind™ Flex FD Solution.

Component	Midi Blot	Mini Blot	Vertically Cut Strip
1X iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
1° Antibody	Dilute antibody to manufacturer's recommended dilution.		

Prepare secondary antibody solution

Dilute antibodies with 1X iBind™ Flex FD Solution.

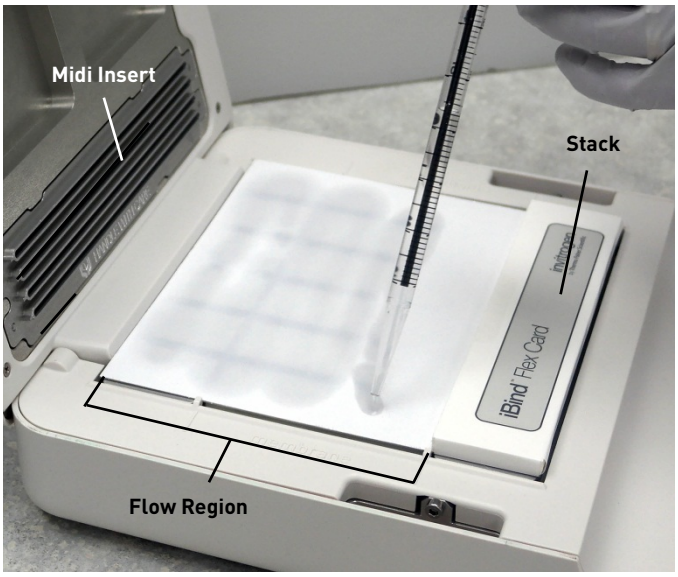
SDS is added to a final concentration of 0.05% to reduce background signal, particularly when using PVDF membranes, or IRDye® 680LT secondary antibodies.

Component	Midi Blot	Mini Blot	Vertically Cut Strip
1X iBind™ Flex FD Solution	4 mL	2 mL	0.7 mL
iBind™ Flex FD 10% SDS	20 µL	10 µL	3.5 µL
Alexa Fluor® 680 **OR** IRDye® 680LT	2 µL (1:2000) 1 µL (1:4000)	1 µL (1:2000) 0.5 µL (1:4000)	0.35 µL (1:2000) 0.18 µL (1:4000)
Alexa Fluor® 790 **OR** IRDye® 800CW	2 µL (1:2000) 1.3 µL (1:3000)	1 µL (1:2000) 0.67 µL (1:3000)	0.35 µL (1:2000) 0.23 µL (1:3000)

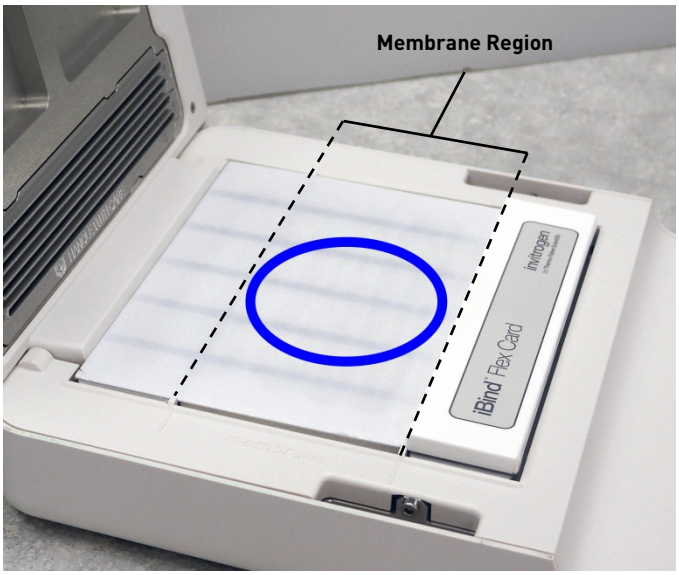
Using the iBind™ Flex Western Device with the Midi Insert

Prepare iBind™ Flex Card

1. Open the lid of the iBind™ Flex Western Device.
2. Verify the Midi Insert is inserted in the iBind™ Flex Western Device.
3. Open the packaging for the iBind™ Flex Card.
4. Hold the iBind™ Flex Card by the Stack, and remove the card from the packaging.
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.

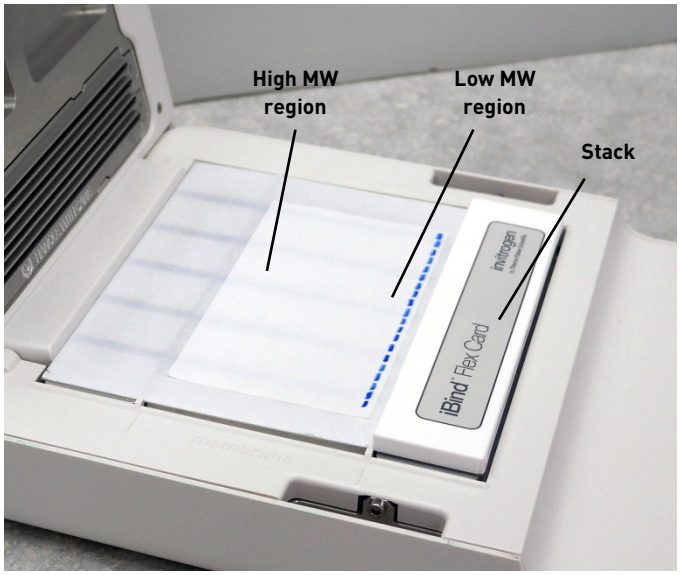


7. Pipette 2 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution so that it pools at the center of the membrane region on the iBind™ Flex Card.

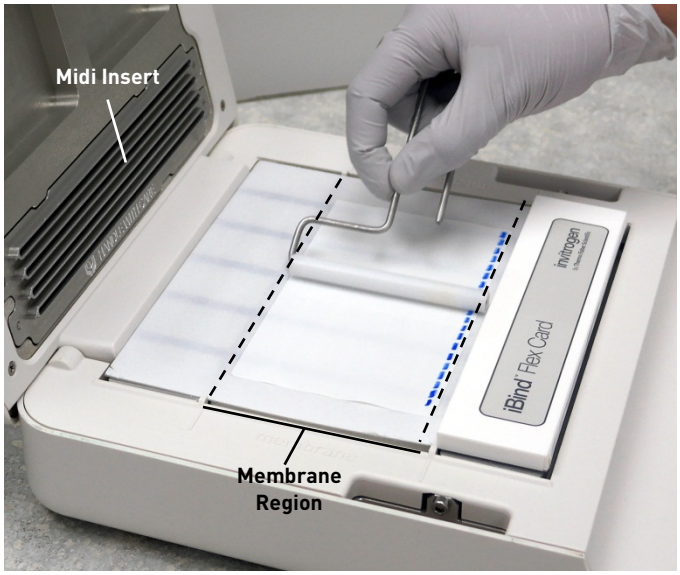


Place membrane on iBind™ Flex Card

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 remove any air bubbles between the membrane and the iBind™ Flex Card.



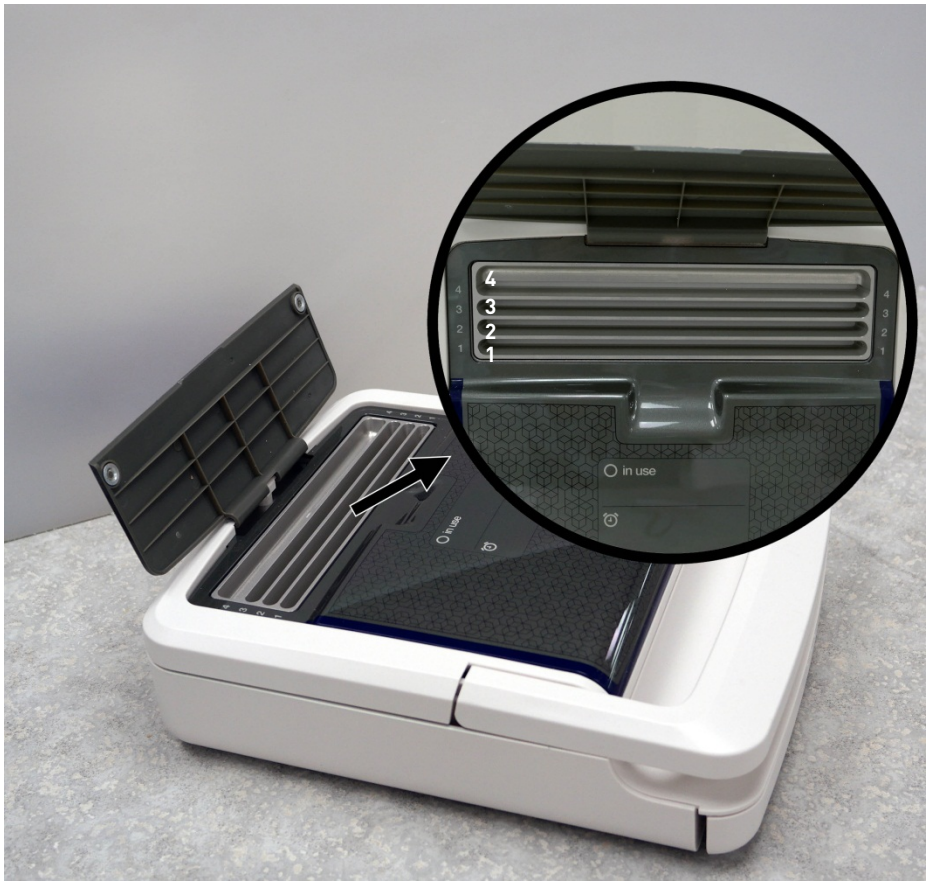
3. Make sure that the the membrane is within the boundaries of the membrane region. **No part of the membrane should be directly under the Midi Insert**.
4. Lower the lid of the iBind™ Flex Western Device and close the latch handle to lock the lid.

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	Volume/Well
1	Diluted primary antibody*	4 mL
2	1X iBind™ Flex/ iBind™ Flex FD Solution	4 mL
3	Diluted secondary antibody	4 mL
4	1X iBind™ Flex/ iBind™ Flex FD Solution	12 mL

* Antibodies can be multiplexed for fluorescent detection (see page 16 for details).



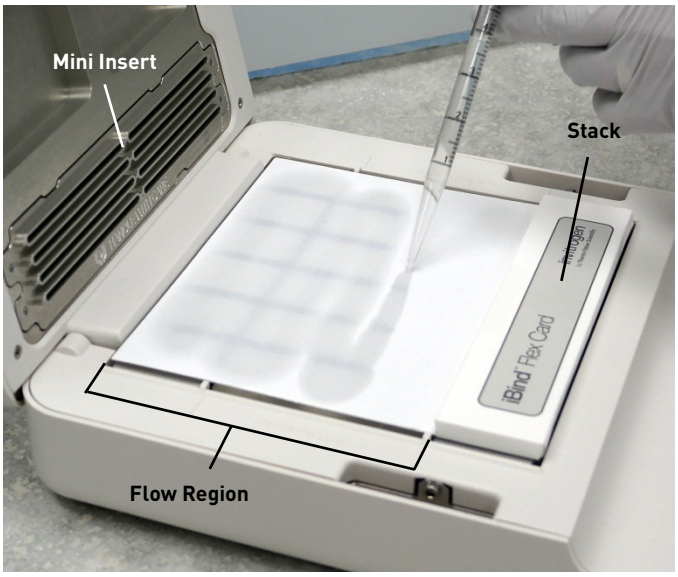
2. Close the Well Cover and write the start time of incubation on the lid of the iBind™ Flex Western device.
3. Incubate 2.5 hours or longer.

   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 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 appropriate detection protocol (page 26–27).
6. Discard iBind™ Flex Card after use.

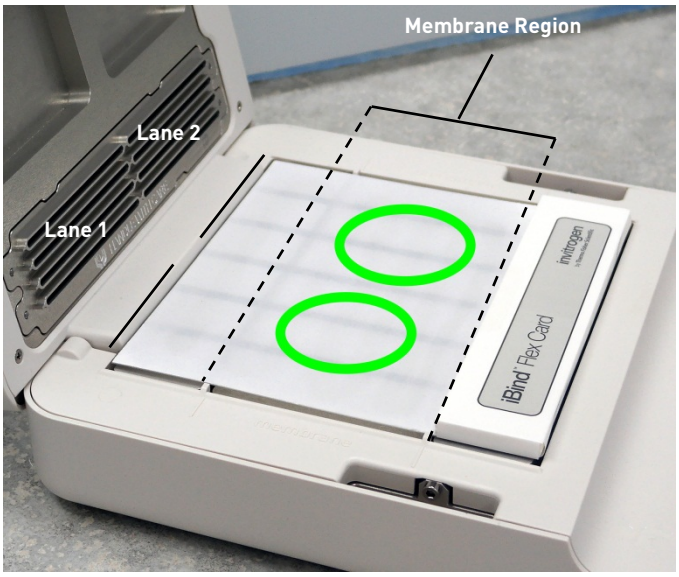
Using the iBind™ Flex Western Device with the Mini Insert

Prepare iBind™ Flex Card

1. Open the lid of the iBind™ Flex Western Device.
2. Verify the Mini Insert is inserted in the iBind™ Flex Western Device.
3. Open the packaging for the iBind™ Flex Card.
4. Hold the iBind™ Flex Card by the Stack, and remove the card from the packaging.
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.

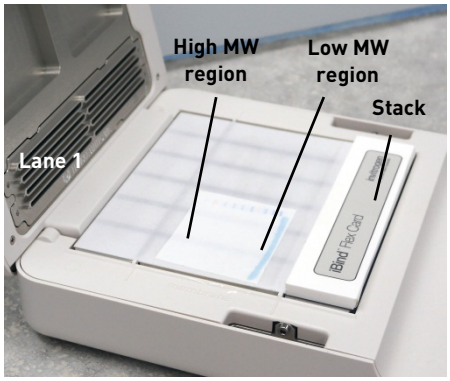
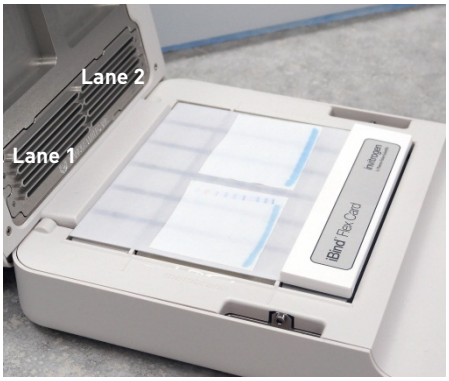


7. Pipette 1 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution in each lane to be used for a membrane so that it pools at the membrane region on the iBind™ Flex Card.

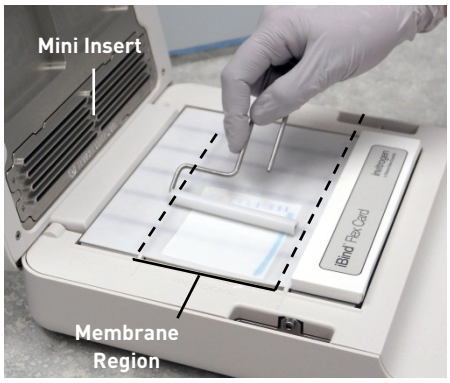
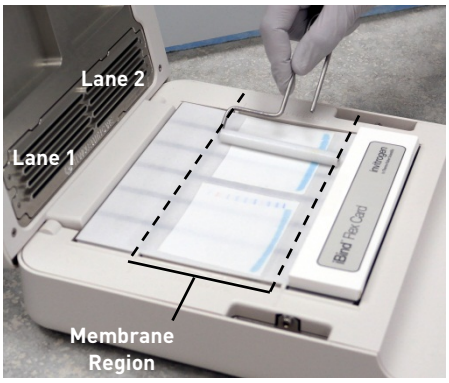


Place membrane on iBind™ Flex Card

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.

One mini membrane in lane 1	Two mini membranes in lanes 1 and 2
	

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

One mini membrane in lane 1	Two mini membranes in lanes 1 and 2
	

3. Make sure that the 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 Mini 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*	Volume/Well
1	Diluted primary antibody**	2 mL
2	1X iBind™ Flex/ iBind™ Flex FD Solution	2 mL
3	Diluted secondary antibody	2 mL
4	1X iBind™ Flex/ iBind™ Flex FD Solution	6 mL

***** 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.

** A different antibody can be used for each lane. Antibodies can also be multiplexed for fluorescent detection (see page 16 for details).

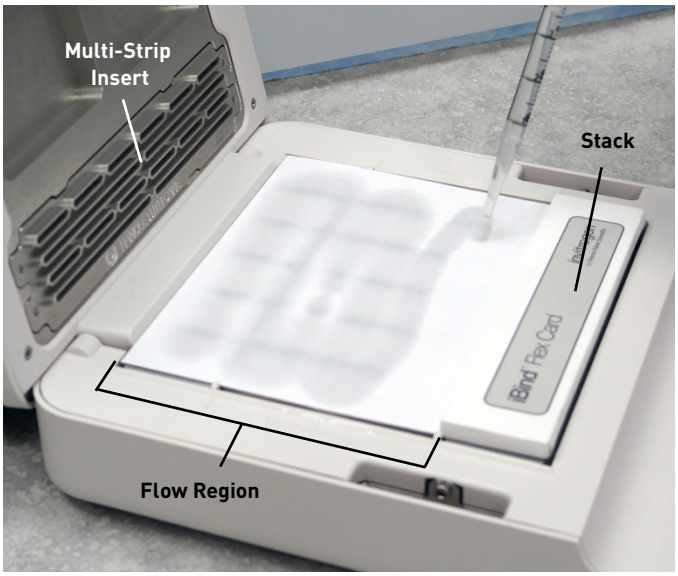
2. Close the Well Cover and write the start time of incubation on the lid of the iBind™ Flex Western device.
3. Incubate 2.5 hours or longer.

   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, and proceed to the appropriate detection protocol (page 26–27).
6. Discard iBind™ Flex Card after use.

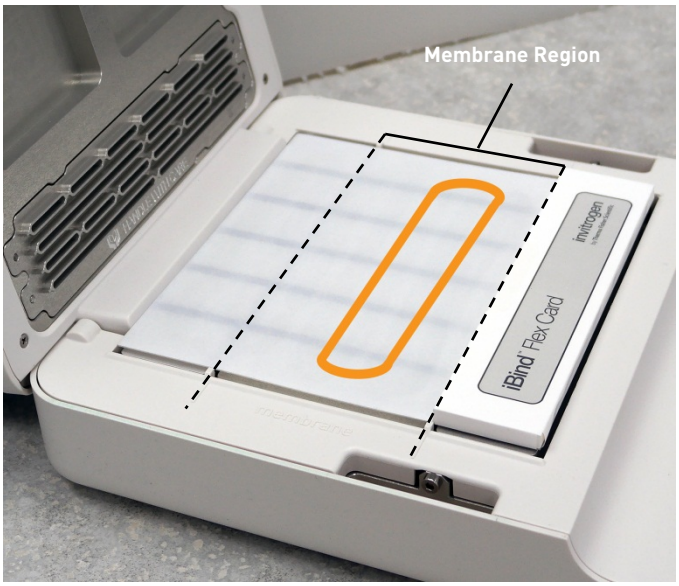
Using the iBind™ Flex Western Device with the Multi-Strip Insert

Prepare iBind™ Flex Card

1. Open the lid of the iBind™ Flex Western Device.
2. Verify the Multi-Strip Insert is inserted in the iBind™ Flex Western Device.
3. Open the packaging for the iBind™ Flex Card.
4. Hold the iBind™ Flex Card by the Stack, and remove the card from the packaging.
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.



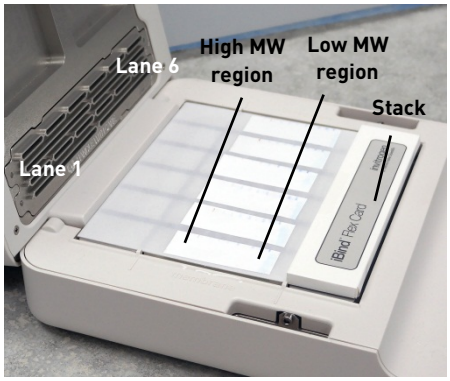
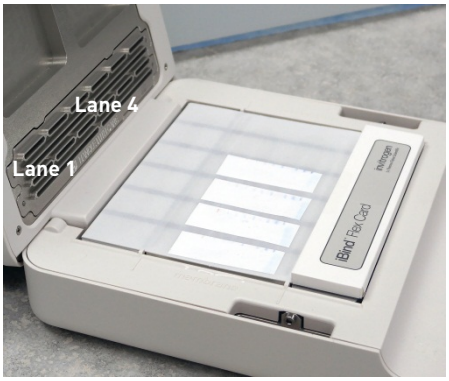
7. Pipette 2 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution so that it pools along the center of the membrane region on the iBind™ Flex Card.



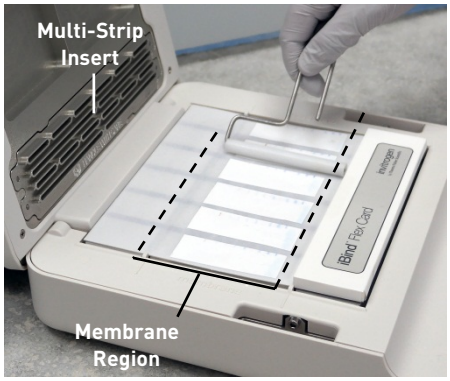
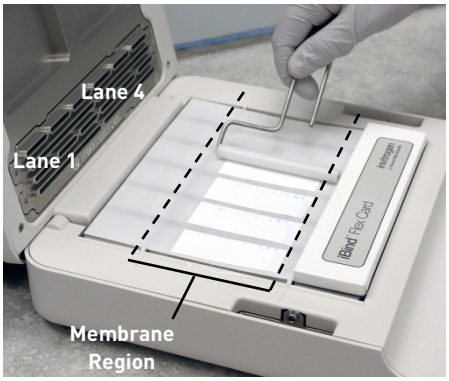
Place membrane on iBind™ Flex Card

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 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 remove any air bubbles between the membrane and the iBind™ Flex Card.

Membranes in all lanes	Membranes in some lanes
	

3. Make sure that the 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*	Volume/Well
1	Diluted primary antibody**	0.7 mL
2	1X iBind™ Flex/ iBind™ Flex FD Solution	2 mL
3	Diluted secondary antibody	0.7 mL
4	1X iBind™ Flex/ iBind™ Flex FD Solution	6 mL

***** 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).

** A different antibody can be used for each lane. Antibodies can also be multiplexed for fluorescent detection (see page 16 for details).

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, and proceed to the appropriate detection protocol (page 26–27).
6. Discard iBind™ Flex Card after use.

Detection

Chemiluminescent detection with alkaline-phosphatase

The following protocol describes performing chemiluminescent detection on a membrane using the Novex® AP Chemiluminescent Detection Kit after blocking, antibody binding, and washes have been completed by the iBind™ Flex Western Device.

1. Prepare chemiluminescent substrate solution based on the type of membrane being used.

   Scale volumes according to the amount needed for the type of membrane being processed.

Solution	Nitrocellulose	PVDF
Novex® AP Chemiluminescent Substrate (CDP-Star®)	2.375 mL	2.5 mL
Chemiluminescent Substrate Enhancer (Nitro-Block-II™)	0.125 mL	—

2. Place the membrane on a sheet of transparency plastic with the **protein-side up**. Do not allow the membrane to dry out.
3. With a clean pipette, apply the appropriate volume of chemiluminescent substrate solution evenly across the membrane surface (do not touch the membrane surface with the pipette).
4. Incubate for 5 minutes.
5. Blot excess chemiluminescent substrate solution from the membrane surface with filter paper. Do not allow the membrane to dry out.
6. Cover the membrane with another clean piece of transparency plastic, or plastic wrap.
7. Place a piece of X-ray film over the membrane sandwich and expose for 1 second to several minutes, then develop the X-ray film,

   OR

   Scan the membrane sandwich in a digital imager.

Chemiluminescent detection with horse-radish peroxidase

The following protocol describes performing chemiluminescent detection on a membrane using the Novex® ECL Chemiluminescent Substrate Reagent Kit after blocking, antibody binding, and washes have been completed by the iBind™ Flex Western Device.

Light emission is most intense from 5–30 minutes after membrane incubation and decreases slowly with time over the course of several hours.

1. Prepare chemiluminescent substrate solution.

   Scale volumes according to the amount needed for the type of membrane being processed.

Solution	Volume
HRP Chemiluminescent Substrate, Reagent A	1.25 mL
HRP Chemiluminescent Substrate, Reagent B	1.25 mL

2. Place the membrane on a sheet of transparency plastic with the **protein-side up**. Do not allow the membrane to dry out.
3. With a clean pipette, apply the appropriate volume of chemiluminescent substrate solution evenly across the membrane surface (do not touch the membrane surface with the pipette).
4. Incubate for 1 minute.
5. Blot excess chemiluminescent substrate solution from the membrane surface with filter paper. Do not allow the membrane to dry out.
6. Cover the membrane with another clean piece of transparency plastic, or plastic wrap.
7. Place a piece of X-ray film over the membrane sandwich and expose for 1 second to several minutes, then develop the X-ray film,

   OR

   Scan the membrane sandwich in a digital imager.

Fluorescent detection

The following protocol describes performing fluorescent detection on a membrane after blocking, antibody binding, and washes have been completed by the iBind™ Flex Western Device.

1. Rinse the membrane with water after completion of the run.
2. Scan the membrane (wet or dry) with the LI-COR® Odyssey® CLx imager using the appropriate channel on "Auto" resolution.

Optimization

Chemiluminescent detection

After performing an initial experiment, conditions can be optimized by varying the dilution of primary and secondary antibodies according to the following table:

Condition/Observation	Primary and Secondary Antibody Concentrations (HRP Protocol)	
	Primary	Secondary
Low signal	1X–5X*	5X
High background with strong signal	1X	1X–5X**

Condition/Observation	Secondary Antibody Concentration (Novex® AP Chemiluminescent Detection Kit)	
	Anti-rabbit	Anti-mouse
Low signal	1:1000 to 1:500	1:500 to 1:250
High background with strong signal	≥1:4000	≥1:2000

* Start with a 1X concentration of primary antibody. Further optimization by increasing the primary antibody concentration may be necessary depending upon your results.

** Start with a 5X concentration of secondary antibody. Further optimization by decreasing the secondary antibody concentration may be necessary depending upon your results.

Fluorescent detection

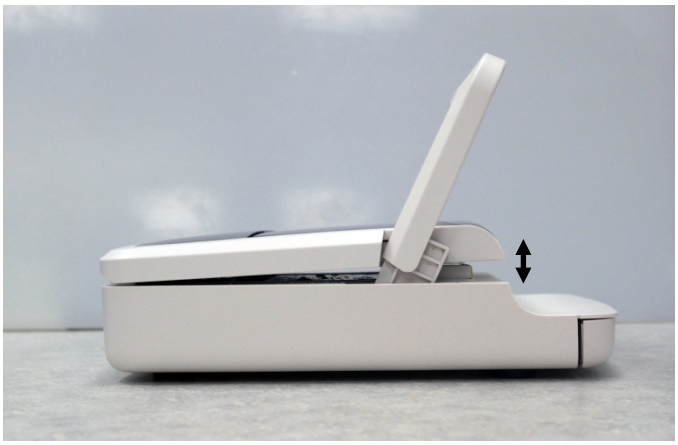
The Standard 1X iBind™ Flex FD Solution is recommended for use with most primary antibodies, and should be used for all initial experiments.

- If initial results give low sensitivity, switch from the Standard 1X iBind™ Flex FD Solution to the Optional 1X iBind™ Flex FD Solution (page15).
- If low sensitivity persists, increase the primary antibody concentration from 1X–5X as needed.

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:



Troubleshooting

Observation	Possible Cause	Solution
Run Times in Excess of 3 hours	iBind™ Flex Card damaged	Replace with new card. Ensure that rolling of the membrane on the card is limited to the area labeled "membrane".
	Stack wet prior to run	Ensure that 10 mL of 1X iBind™ Flex/iBind™ Flex FD Solution is added to the flat region of the iBind™ Flex 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 (pages 13, 15).
High background	Membrane 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.
	Membrane is contaminated	Use only clean, new membranes. Wear clean gloves at all times and use forceps when handling membranes.
	Film 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 is 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.
	Concentrated primary antibody used	Follow the supplier's recommended dilution or determine the optimum concentration by dot blotting.
	Incorrect chemiluminescent substrate used for PVDF	Make sure CDP-Star without enhancer is used.
	Blot is overdeveloped	Follow recommended developing time or remove blot from substrate when signal-to-noise ratio is acceptable.
	Ink 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 (pages 13, 15).
	Improper application of solutions to iBind™ Flex Wells	Add the appropriate solutions for each well in numerical order (pages 19, 22, 25).

Observation	Possible Cause	Solution
High background, continued	Blot improperly placed on iBind™ Flex Card	• Place the membrane in the designated Membrane Region on the iBind™ Flex Card. • The protein side of the blot should be in contact with the iBind™ Flex Card. • The low MW regions should be closest to the Stack. • The membrane should not be in contact with the Stack.
	Card stack 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.
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed (pages 13, 15).
	Improper application of solutions to iBind™ Flex Wells	Add the appropriate solutions for each well in numerical order (pages 19, 22, 25).
	Blot improperly placed on iBind™ Flex Card	• Place the membrane in the designated Membrane Region on the iBind™ Flex Card. • The protein side of the blot should be in contact with the iBind™ Flex Card. • The low MW regions should be closest to the Stack. • The membrane should not be in contact with the Stack.
	Card stack 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.
Non-specific binding	Membrane contaminated by fingerprints or keratin proteins	Wear clean gloves at all times and use forceps when handling membranes. Always handle membranes around the edges.
	Primary antibody too concentrated	Follow the supplier's recommended dilution or determine the optimum concentration by dot blotting.
	Insufficient removal of SDS/weakly bound proteins from membrane after blotting	Follow instructions for membrane preparation before immunodetection (pages 13, 15).
	Affinity of the primary antibody for the protein standards	Check with protein standard manufacturer for homologies with primary antibody.
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed (pages 13, 15).

Observation	Possible Cause	Solution
Weak or no signal	Poor or incomplete transfer	Refer to Western Blotting instructions (IM-9051) and repeat blot. After blotting, stain membrane to measure transfer efficiency. Use positive control and/or molecular weight marker.
	Membrane not completely wet	Follow instructions for pre-wetting the membrane.
	Primary antibody concentration too low	Follow the supplier's recommended dilution or determine the optimum concentration by dot blotting.
	Inactive primary antibody	Determine activity by performing a dot-blot.
	Low affinity of primary antibody to antigen	Obtain a higher affinity primary antibody.
	Contaminated secondary antibody solution	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 (see Western Blotting instructions).
	Improper preparation of iBind™ Flex/iBind™ Flex FD Solution	Prepare 1X iBind™ Flex/iBind™ Flex FD Solutions as directed (pages 13, 15).
	Improper application of solutions to iBind™ Flex Wells	Ensure that the solutions are added to the correct wells and that the wells are loaded in numerical order.
	Blot improperly placed on iBind™ Flex Card	Ensure that the protein side of the blot is in contact with the iBind™ Flex Card and is placed in the region labeled “membrane”.
	Stack wet prior to run	Ensure that 10 mL of 1X iBind™ Flex/iBind™ Flex FD Solution is added to the flat region of the iBind™ Card. Avoid adding solution to the Stack.
	Cross-contamination of solutions in wells	Do not move the iBind™ Flex Western Device during the run.
	iBind™ Flex Card damaged	Replace with new card. 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 a 10 mL pool of 1X iBind™ Flex/iBind™ Flex FD Solution. Use the roller provided to ensure proper contact.

Observation	Possible Cause	Solution
Weak or no signal, continued	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.
	Sample improperly prepared; antigenicity compromised	SDS and reducing agents may interfere with some antibody/antigen affinities.
	Sample too dilute	Load a higher concentration or amount of protein onto the gel.
	Protein weakly bound to membrane	Ensure that transfer buffer contains 10–20% methanol.
	Insufficient exposure time	Re-expose film for a longer period of time.
	Insufficient substrate incubation	Perform each step for the specified amount of time or remove blot from substrate when signal-to-noise ratio is acceptable.
	Substrate is contaminated	Wear gloves at all times and keep bottles tightly capped when not in use.
	Blots are too old	Protein may have broken down over time. Use freshly prepared blots.
Large, scattered signal	Protein is overloaded	Reduce load or dilute concentration of sample.
	Poor or incomplete transfer	Refer to Western Blotting instructions (IM-9051) and repeat blot.
	Primary antibody is too concentrated	Follow the supplier's recommended dilution or determine the optimum concentration by dot blotting.
"Spotted" membrane	Poor or incomplete transfer	Refer to Western Blotting instructions (IM-9051) and repeat blot.
	Membrane pads are dirty or contaminated	Soak with detergent and rinse thoroughly with purified water before use. Replace pads when they become worn or discolored.
	Membrane not completely wet	Follow instructions for pre-wetting the membrane.
	Membrane is contaminated by fingerprints or keratin proteins	Wear clean gloves at all times and use forceps when handling membranes. Always handle membranes around the edges.
	Uneven blocking	The incubation dish must be small enough to allow thorough coverage of membrane.
	Ink used to label membrane	Any labeling of the membrane with ink should be limited to the low MW region of the blot.
	iBind™ Flex Card damaged	Replace with new card. Ensure that rolling of the membrane on the card is limited to membrane region.
	Membrane is not in proper contact with the iBind™ Flex Card	Place the membrane on the iBind™ Flex Card immediately after wetting the card with 10 mL of 1X iBind™ Flex/iBind™ Flex FD Solution. Use the roller provided to ensure proper contact.

Appendix A

Related Products

Additional products

Many of the components of the iBind™ Flex Western System, as well as additional reagents that may be used for electrophoresis of proteins are available separately from Life Technologies. Ordering information is provided below. For details, visit **www.lifetechnologies.com** or call Technical Support (page 35).

Parts	Quantity	Cat. 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 unit	LC2100
AlexaFluor® 680 Goat Anti-Rabbit IgG (H+L)	0.5 mL	A21109
AlexaFluor® 790 Goat Anti-Mouse IgG (H+L)	0.5 mL	A11375
Novex® AP Mouse Chemiluminescent Detection Kit	1 kit	SLF1021
Novex® AP Rabbit Chemiluminescent Detection Kit	1 kit	SLF1022
Goat Anti-Mouse IgG (H+L) - HRP	1 mL	62-6520
Goat Anti-Rabbit IgG - HRP	1 mL	65-6120
Novex® ECL Chemiluminescent Substrate Reagent Kit	2 × 125 mL	WP20005
iBlot® 2 Gel Transfer Device	1 device	IB21001
iBlot® 2 Regular Transfer Stack, Nitrocellulose	10 stacks	IB23001
iBlot® 2 Regular Transfer Stack, PVDF	10 stacks	IB24001
iBlot® 2 Mini Transfer Stack, Nitrocellulose	10 stacks	IB23002
iBlot® 2 Mini Transfer Stack, PVDF	10 stacks	IB24002
Mini Gel Tank	1 unit	A25977
Bolt® 4-12% Bis-Tris Plus Gel, 10 Well	10 gels	BG04120BOX
NuPAGE® Novex® 4-12% Bis-Tris Gels, 1.0 mm, 10 well	10 gels	NP0321BOX
NuPAGE® Novex® 4-12% Bis-Tris Gels, 1.5 mm, 10 well	10 gels	NP0335BOX

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available from www.lifetechnologies.com

Limited warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at http://www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies.

Appendix B

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 (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene) or acetone.

iBind™ Flex Card specifications

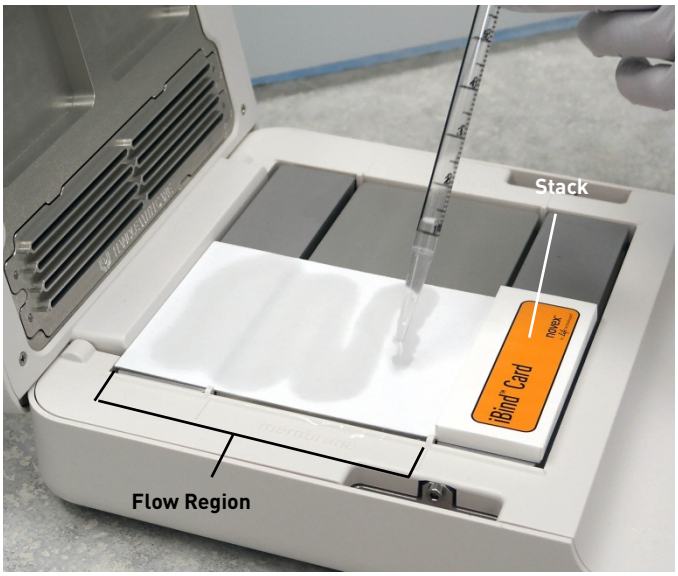
Dimensions:	17.8 cm (l) × 17.8 cm (w) × 0.8 cm (stack height)
Material:	Glass fiber
Operating Temperature:	18˚C to 30˚C
Temperature Limit:	30°C

Appendix C

Using the iBind™ Flex Western Device with an iBind™ Card

Prepare iBind™ Card

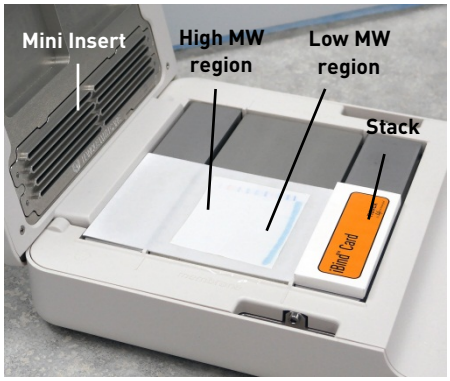
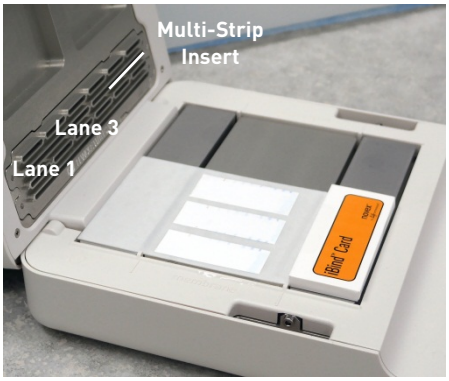
1. Open the lid of the iBind™ Flex Western Device.
2. Verify the Mini Insert, or Multi-Strip Insert is inserted in the iBind™ Flex Western Device (depending upon the type of blot being processed).
3. Open the packaging for the iBind™ Card.
4. Hold the iBind™ Card by the Stack, and remove the card from the packaging.
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.



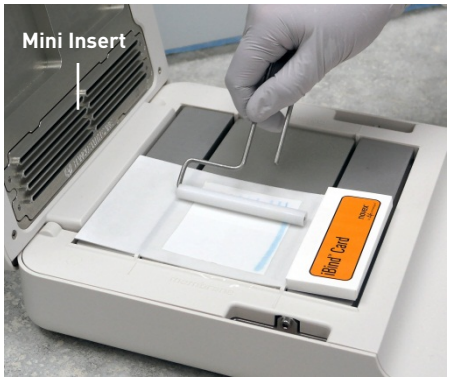
7. Pipette 1 mL of 1X iBind™ Flex/ iBind™ Flex FD Solution so that it pools at the center of the membrane region on the iBind™ Card.

Place membrane on 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 remove any air bubbles between the membrane and the iBind™ Card.

Mini size membrane	Vertically cut strips
	

3. Make sure that 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 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*	Volume/Well	
		Mini Blot	Vertically Cut Strip(s)
1	Diluted primary antibody	2 mL	0.7 mL
2	1X iBind™ Flex/ iBind™ Flex FD Solution	2 mL	2 mL
3	Diluted secondary antibody	2 mL	0.7 mL
4	1X iBind™ Flex/ iBind™ Flex FD Solution	6 mL	6 mL

* 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.

Mini Insert	Multi-Strip Insert
	

2. Close the Well Cover and 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.

3. 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.

4. After incubation, rinse the membrane twice in 20 mL of distilled water for 2 minutes, and proceed to the appropriate detection protocol (page 26–27).

5. Discard iBind™ Card after use.

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

thermofisher.com/lifescience

13 June 2015

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