

**Prepare Antibodies**

Dilute antibodies with 1X iBind™ Flex Solution.

Component	Midi Blot	Mini Blot	Vertical Strip
<b>Primary Antibody (1° Ab) Solution</b>			
1X iBind™ Flex Solution	4 mL	2 mL	0.7 mL
1° Antibody	Use final antibody concentration equal to the manufacturer's recommended dilution		
<b>Secondary Antibody (2° Ab) Solution</b>			
1X iBind™ Flex Solution	4 mL	2 mL	0.7 mL
2° Antibody	Use final antibody concentration at 5X the manufacturer's recommended dilution. (e.g. 1:1000 dilution if 1:5000 dilution recommended)		

**Disclaimer**

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 <b>Package Contents</b>	<p><b>Cat. No.</b> SLF2020</p> <p><b>Size</b> 10 midi rxns</p> <ul style="list-style-type: none"> <li>▪ iBind™ Flex 5X Buffer</li> <li>▪ iBind™ Flex 100X Additive</li> </ul>
 <b>Storage Conditions</b>	<ul style="list-style-type: none"> <li>▪ Store buffer and additive at 4°C (do not freeze)</li> </ul>
 <b>Required Materials</b>	<ul style="list-style-type: none"> <li>▪ iBind™ Flex Western Device</li> <li>▪ iBind™ Flex Card</li> <li>▪ Blotting roller</li> <li>▪ Appropriate HRP or AP conjugated secondary antibody</li> <li>▪ Chemiluminescent detection kit</li> </ul>
 <b>Timing</b>	<p>Preparation time: ~10 minutes</p> <p>Run time: Allow row 4 to empty; ~2.5 hours or longer</p>
 <b>Product Description</b>	<ul style="list-style-type: none"> <li>▪ This iBind™ Flex Solution Kit facilitates primary and secondary antibody binding of membrane-bound proteins in western detection.</li> </ul>
 <b>Important Guidelines</b>	<ul style="list-style-type: none"> <li>▪ Wear gloves when handling the iBind™ Flex card.</li> <li>▪ Do not open the iBind™ Flex device after closing it over an iBind™ Flex card.</li> </ul>
 <b>Online Resources</b>	<p>Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a>.</p>

Online Specials

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

## iBind™ Flex Solution Kit Protocol

Follow the instructions below to prepare solutions and perform antibody binding for 1 midi, 2 mini, or up to 6 vertically cut strip blots with the iBind™ Flex Western Device.

Timeline	Steps	Procedure Details																				
1	 Prepare 1X Flex Solution	Prepare 1X iBind™ Flex Solution for HRP and AP detection protocols.																				
		<table border="1"> <thead> <tr> <th>Component</th> <th>1X iBind™ Flex Solution</th> </tr> </thead> <tbody> <tr> <td>iBind™ Flex 100X Additive</td> <td>500 µL</td> </tr> <tr> <td>iBind™ Flex 5X Buffer</td> <td>10 mL</td> </tr> <tr> <td>Distilled water</td> <td>39.5 mL</td> </tr> </tbody> </table>	Component	1X iBind™ Flex Solution	iBind™ Flex 100X Additive	500 µL	iBind™ Flex 5X Buffer	10 mL	Distilled water	39.5 mL												
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2	 Prepare diluted antibodies	See "Prepare Antibodies" for details.																				
3	 Load iBind™ Flex card and membrane in iBind™ Flex device	<ol style="list-style-type: none"> <li>1. Immerse the membrane in 10 mL of 1X iBind™ Flex Solution.</li> <li>2. Place the iBind™ Flex card on the iBind™ Flex device. Ensure the card is aligned with the stoppers.</li> <li>3. Apply 10 mL 1X iBind™ Solution to the card so that the Flow Region is completely wet.</li> <li>4. Add 1X iBind™ Solution to the Membrane Region (see User Guide or Quick Reference for details).</li> <li>5. Place the blot on the card, <b>protein side down</b>, with the low MW region toward the stack.</li> <li>6. Remove any air bubbles between the iBind™ Flex card and the membrane with a blotting roller.</li> </ol>																				
4	 Add prepared antibody and wash solutions	<table border="1"> <thead> <tr> <th>Add solutions in the following order:</th> <th>Midi Blot</th> <th>Mini Blot</th> <th>Vertical Strip</th> </tr> </thead> <tbody> <tr> <td>Row 1: diluted 1° antibody</td> <td>4 mL/well</td> <td>2 mL/well</td> <td>0.7 mL/well</td> </tr> <tr> <td>Row 2: 1X iBind™ Flex Sol'n</td> <td>4 mL/well</td> <td>2 mL/well</td> <td>2 mL/well</td> </tr> <tr> <td>Row 3: diluted 2° antibody</td> <td>4 mL/well</td> <td>2 mL/well</td> <td>0.7 mL/well</td> </tr> <tr> <td>Row 4: 1X iBind™ Flex Sol'n</td> <td>12 mL/well</td> <td>6 mL/well</td> <td>6 mL/well</td> </tr> </tbody> </table>	Add solutions in the following order:	Midi Blot	Mini Blot	Vertical Strip	Row 1: diluted 1° antibody	4 mL/well	2 mL/well	0.7 mL/well	Row 2: 1X iBind™ Flex Sol'n	4 mL/well	2 mL/well	2 mL/well	Row 3: diluted 2° antibody	4 mL/well	2 mL/well	0.7 mL/well	Row 4: 1X iBind™ Flex Sol'n	12 mL/well	6 mL/well	6 mL/well
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5	 Run	Close the iBind™ Flex device well cover to minimize evaporation. Leave the device undisturbed until the well(s) of row 4 are empty (~2.5 hours or longer).																				
6	 Detect blot	Rinse the membrane in distilled water. Perform HRP or AP chemiluminescent detection according to manufacturer recommendations.																				