

# Technical Note

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## One Blot Western Optimization Using the MPX™ Blotting System

Developed for:

**Aerius, and Odyssey®  
Family of Imaging Systems**

*Please refer to your manual to confirm  
that this protocol is appropriate for the  
applications compatible with your  
Odyssey Imager model.*



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### I. INTRODUCTION

The independent channels of the LI-COR® MPX (Multiplex) Blotter facilitate the ability to optimize blocking buffer, primary antibody dilution, and secondary antibody dilution in a single Western blot. Western blotting procedures that generate a blot of 7.0 × 8.5 cm are easily adaptable to the MPX format. The process fits into any laboratory's standard Western blot work flow. Both self-poured and pre-cast gels can be used to generate blots. Run electrophoresis and transfer to standard nitrocellulose or PVDF membrane under standard conditions. Clamp the blot into the MPX Blotter, which creates up to 24 independent channels. The range of usable channels per sample is relative to comb size. For Western Blot optimization, a single-well gel is all that is needed. For this application, any detection method can be used, including near infrared and chemiluminescence. The following is a general guideline for use with the Odyssey® Infrared Imaging System.

### II. SUGGESTED MATERIALS

	Reagent/Supply	LI-COR P/N
<b>Sample Preparation</b>	4X Protein Sample Loading Buffer	928-40004
<b>Electrophoresis</b>	Odyssey One-Color Protein Markers (Molecular Weight - 10 kDa to 250 kDa)	928-40000
<b>Blotting and Transfer</b>	10X Tris Glycine (liquid or powder)	928-40010 or 928-40012
	Odyssey Nitrocellulose (7 x 8.5 cm or roll)	926-31090 or 926-31092
<b>MPX Detection</b>	LI-COR Blocking Buffer	927-40050
	<i>Sample Pack:</i>	
	• Odyssey Blocking Buffer	
	• Casein Blocking Buffer	
	Commercial Milk Blocking Buffer	
	IRDye® Labeled Secondary Antibodies	Multiple P/Ns
10X PBS (liquid or powder)	928-40018 or 928-40020	
MPX Membrane Cushion	921-00120	
<b>Imaging</b>	Odyssey Infrared Imaging System	

### III. GEL ELECTROPHORESIS & TRANSFER

- **Gel Preparation**

There are a wide variety of gel matrices that are compatible with the MPX Blotter detection system. LI-COR® provides a solution for pouring gels with the NEXT GEL System (Amresco). The gel matrix can be used in your gel casting system with a single-well comb such as Bio-Rad (mini PROTEAN Comb, prep/2-D well, P/N 165-3361 1.0 mm, or P/N 165-3367, 1.5 mm) or from a vendor of the user's choice. Alternatively, pre-cast gels can be purchased and used. See Table 1 for a list of pre-cast gels available, and the number of usable ports compatible with the MPX Blotter.

**Table 1.** Single sample pre-cast gel options for use with the MPX Blotter.

Vendor	Well Designation	Sample #	MW Marker Well	Usable Ports
Invitrogen	2D	1	Yes	19
Bio-Rad	2D/Prep	1	Yes	21
C.B.S. Scientific	1 Well	1	No	23

- **Sample Preparation**

When using a single-well gel, a larger volume of sample is required. Prepare your protein sample so that the sample volume and concentration is equivalent to running all the lanes on a standard 10-well gel. Example: 5 µg of lysate per lane = 50 µg in a total volume of 100-150 µL, including loading buffer.

The following procedure can be used:

Dilute the sample 1:4 in 4X Protein Sample Loading Buffer (LI-COR P/N 928-40004) with β-Mercaptoethanol. See pack insert at [www.licor.com/bio/reagents/buffers.jsp](http://www.licor.com/bio/reagents/buffers.jsp) for detailed instructions. Heat the sample at 95°C for 5 minutes.

- **Molecular Weight Marker**

It is important to have a molecular weight marker that is visible to the eye because the marker is the primary tool used to align the blot in the MPX Blotter. Odyssey One-Color Molecular Weight Markers (LI-COR P/N 928-40000) is the recommended marker choice.

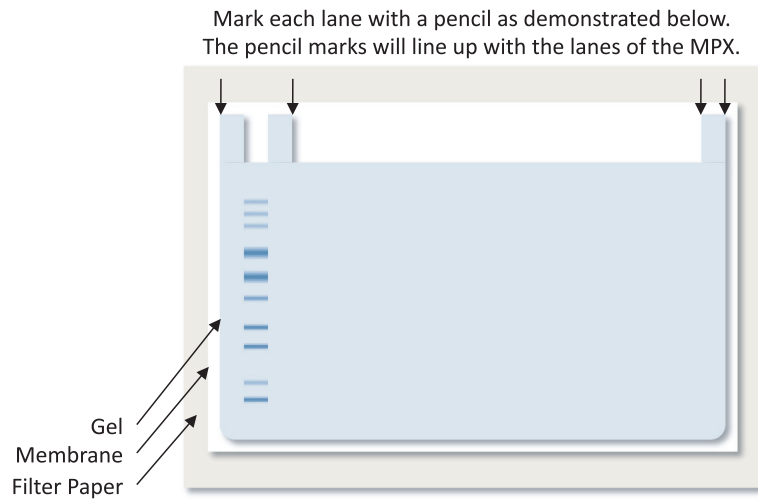
- **Electrophoresis**

**Important:** The maximum length of the separating gel should not exceed 50 mm—the length of the channels on the MPX Blotter.

- **Transfer**

Always use clean forceps when handling membranes. Once electrophoresis is complete, transfer proteins to Odyssey® Nitrocellulose Membrane (LI-COR® P/N 926-31092 or 926-31090) using standard transfer procedures. Mark the outside corners of the gel and sample wells with a pencil before separating the transferred gel from the membrane as in Figure 1. The marks help align the membrane once it is placed on the MPX Blotter. Allow the membrane to dry a minimum of one hour before proceeding with detection.

**Important:** Ink from most pens will fluoresce on the Odyssey Imager.

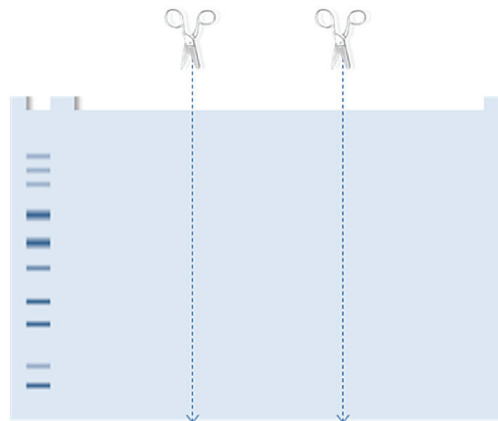


**Figure 1.** Diagram showing how to effectively mark the membrane for alignment into the MPX Blotter.

#### IV. MEMBRANE BLOCKING

- **Membrane Preparation**

Cut the membrane into three individual blots as shown in Figure 2. Pre-wet membranes in PBS before proceeding with blocking.



**Figure 2.** Diagram showing how to cut the membrane into three individual blots for blocking buffer optimization.

- **Blocking**

Place the membranes into 3 different incubation boxes. In each box, cover the entire membrane with blocking buffer (approximately 0.4 mL/cm<sup>2</sup>), using a different blocking buffer for each membrane. Block the membrane for 1 hour at room temperature.

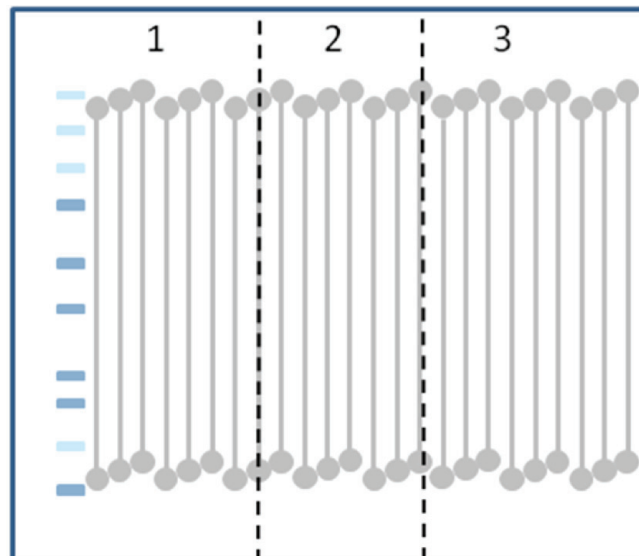
Example:

Membrane	Blocker
1	Odyssey® Blocking Buffer
2	Casein Blocking Buffer
3	Commercial Milk Blocking Buffer

## V. ALIGNMENT IN MPX BLOTTER

For detailed instruction on use of the MPX Blotter, see MPX Blotter Multiplex Western Blotting Accessory User Guide at [http://biosupport.licor.com/docs/MPX\\_Blotter\\_UG\\_10644.pdf](http://biosupport.licor.com/docs/MPX_Blotter_UG_10644.pdf)

Place the three blocked membranes into the MPX Blotter so that there are at least 4 channels available for use on each membrane. See Figure 3.



**Figure 3.** Diagram of how to place three individual blots into the MPX Blotter.

## VI. PRIMARY & SECONDARY ANTIBODY APPLICATION

- **Primary Antibody Preparation**

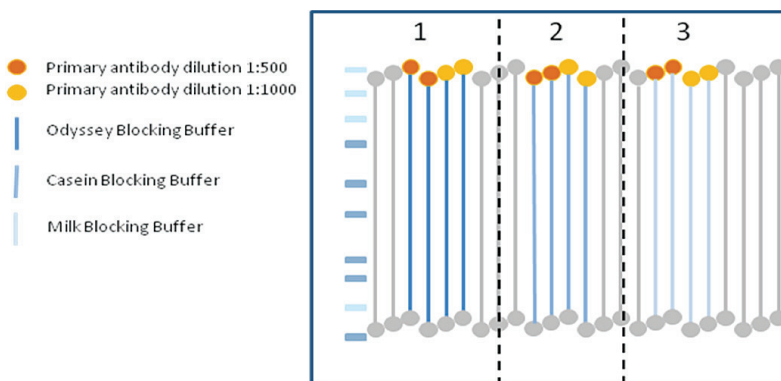
Two dilutions of primary antibody need to be made for each blocking buffer being evaluated. Dilutions should be chosen based on vendor recommendations. 500 µL of each dilution will be needed.

Example:

Membrane	Blocker	Primary Antibody Dilution	
1	Odyssey® Blocking Buffer	1:500	1:1,000
2	Casein Blocking Buffer	1:500	1:1,000
3	Commercial Milk Blocking Buffer	1:500	1:1,000

- **Primary Antibody Application**

The primary antibody/blocker dilutions should be loaded into the MPX Blotter to correspond with the same blocked membrane. Apply 2 replicates of each primary antibody dilution; see Figure 4. Fill the unused channels with appropriate corresponding blocking buffer. Incubate for 1-4 hours at room temperature. Wash primary antibody from the channels thoroughly according to MPX Blotter manual instructions, using 1X PBS-T.



**Figure 4.** Diagram of how to place primary antibodies into the MPX Blotter.

- **Secondary Antibody Preparation**

Two dilutions of secondary antibody need to be made for each blocking buffer being evaluated. Dilutions should be chosen based on vendor recommendations. For IRDye® conjugated secondary antibodies, we recommend 1:5,000 and 1:10,000 as a starting point. 500 µL of each antibody will be needed.

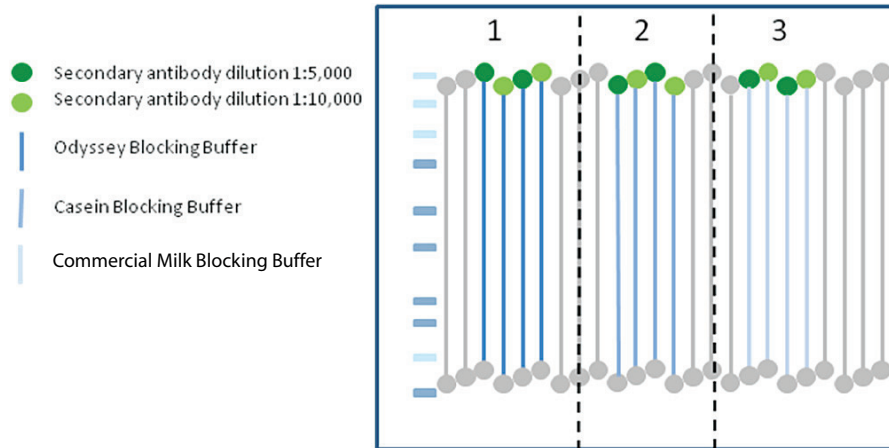
Example:

Membrane	Blocker	Secondary Antibody Dilution	
1	Odyssey Blocking Buffer	1:5,000	1:10,000
2	Casein Blocking Buffer	1:5,000	1:10,000
3	Commercial Milk Blocking Buffer	1:5,000	1:10,000

- **Secondary Antibody Application**

The secondary antibody/blocker dilutions should be loaded into the MPX Blotter to correspond with the same blocked membrane. Add the secondary antibody dilutions to the primary antibody channels; see Figure 5. Fill the unused channels with the appropriate corresponding blocking buffer. Incubate 1 hour at room temperature. Wash secondary antibody from the channels thoroughly using 1X PBS-T, according to MPX Blotter manual instructions.

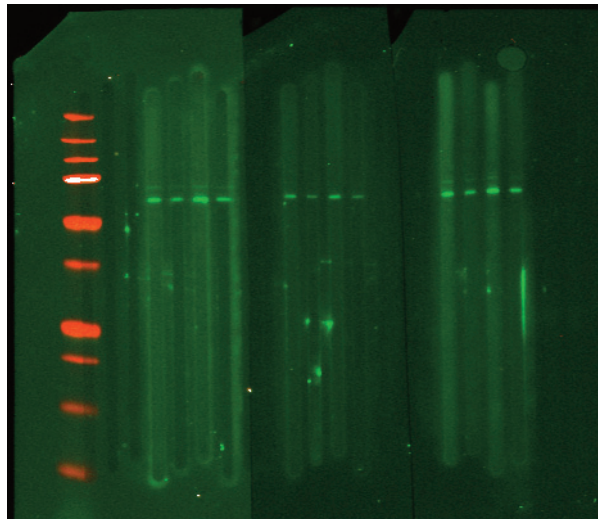
- Blots can be removed from the MPX Blotter and washed in 1X PBS-T for 5 minutes, followed by a 1X PBS rinse.



**Figure 5.** Diagram of how to place secondary antibodies into the MPX Blotter.

## VII. IMAGING

Membranes can be imaged immediately. Use standard Western blot imaging settings on any Odyssey® Imaging System. Example data is shown in Figure 6.



**Figure 6.** Akt antibody optimization using the MPX Blotter procedure.

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44647 Superior St. • P.O. Box 4000 • Lincoln, Nebraska 68504  
LI-COR Biosciences North America: 800-645-4267 / 402-467-0700  
FAX: 402-467-0819 • Technical Support: 800-645-4260

LI-COR GmbH, Germany: Serving Europe, Africa, and the Middle East: +49 (0) 6172 17 17 771

LI-COR Ltd, UK: Serving UK, Ireland and Scandinavia: +44 (0) 1223 422104

In other countries, contact LI-COR Biosciences or a local LI-COR distributor:  
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