

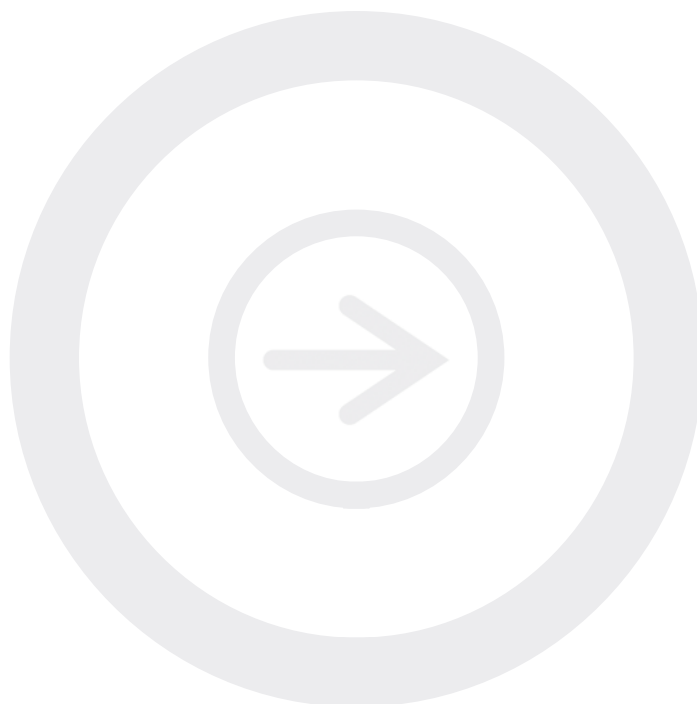
Odyssey Western Blot Blocker Optimization

Developed for:

Aerius and Odyssey® Family of Imagers

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

Part Numbers: 927-40050
 927-40300
 927-40200
 927-40100
 927-40000
 927-40003
 927-40010



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I. Required Reagents

- Odyssey Protein Marker

| Protein Marker | LI-COR P/N |
|---|------------|
| Odyssey Protein Molecular Weight Marker | 928-40000 |

- IRDye Secondary Antibodies

| Dye | | LI-COR P/N |
|-------------|-----------------------------------|------------|
| IRDye 800CW | Goat anti-Mouse | 926-32210 |
| IRDye 800CW | Goat anti-Rabbit | 926-32211 |
| IRDye 800CW | Donkey anti-Mouse | 926-32212 |
| IRDye 800CW | Donkey anti-Rabbit | 926-32213 |
| IRDye 800CW | Donkey anti-Goat | 926-32214 |
| IRDye 800CW | Donkey anti-Chicken | 926-32218 |
| IRDye 800CW | Goat anti-Rat | 926-32219 |
| IRDye 800CW | Donkey anti-Guinea Pig | 926-32411 |
| IRDye 800CW | Goat anti-Human | 926-32232 |
| IRDye 800CW | Goat anti-Mouse IgG ₁ | 926-32350 |
| IRDye 800CW | Goat anti-Mouse IgG _{2a} | 926-32351 |
| IRDye 800CW | Goat anti-Mouse IgG _{2b} | 926-32352 |
| IRDye 680LT | Goat anti-Mouse | 926-68020 |
| IRDye 680LT | Goat anti-Rabbit | 926-68021 |
| IRDye 680LT | Donkey anti-Mouse | 926-68022 |
| IRDye 680LT | Donkey anti-Rabbit | 926-68023 |
| IRDye 680LT | Donkey anti-Goat | 926-68024 |
| IRDye 680LT | Donkey anti-Chicken | 926-68028 |
| IRDye 680LT | Goat anti-Rat | 926-68029 |
| IRDye 680LT | Donkey anti-Guinea Pig | 926-68030 |
| IRDye 680LT | Goat antiHuman | 926-68032 |
| IRDye 680LT | Goat anti-Mouse IgG ₁ | 926-68050 |
| IRDye 680LT | Goat anti-Mouse IgG _{2a} | 926-68051 |
| IRDye 680LT | Goat anti-Mouse IgG _{2b} | 926-68052 |

Continued

- IRDye Secondary Antibodies (Continued)

| Dye | | LI-COR P/N |
|-------------|------------------------|-------------------|
| IRDye 680RD | Goat anti-Mouse | 926-68070 |
| IRDye 680RD | Goat anti-Rabbit | 926-68071 |
| IRDye 680RD | Donkey anti-Mouse | 926-68072 |
| IRDye 680RD | Donkey anti-Rabbit | 926-68073 |
| IRDye 680RD | Donkey anti-Goat | 926-68074 |
| IRDye 680RD | Donkey anti-Chicken | 926-68075 |
| IRDye 680RD | Goat anti-Rat | 926-68076 |
| IRDye 680RD | Donkey anti-Guinea Pig | 926-68077 |
| IRDye 680RD | Goat anti-Human | 926-68078 |

- Blocking Buffer

| Blocking Buffer | Volume | LI-COR P/N |
|------------------------------|---------------|-------------------|
| Blocking Buffer Sample Pack: | | 927-40050 |
| Odyssey Blocking Buffer | 125 mL | |
| Casein Blocking Buffer | | |
| Odyssey Blocking Buffer | 125 mL | 927-40100 |
| | 500 mL | 927-40000 |
| Casein Blocking Buffer | 100 mL | 927-40300 |
| | 500 mL | 927-40200 |

- Membrane

| Membrane | Size | LI-COR P/N |
|---|----------------------|-------------------|
| Odyssey Nitrocellulose (0.22 µm), 10 pack | 7 x 8.5 cm | 926-31090 |
| Odyssey Nitrocellulose (0.22 µm), roll | 30 cm x 3 m | 926-31092 |
| Millipore Immobilon®-FL (0.45 µm) | | |
| Western Blot Kits | 10 x 10 cm (10 pack) | 926-31050 |
| | | 926-31052 |
| Blocking Buffer & Membrane Kit | 26.5 cm x 3.75 m | 829-31080 |

- Primary antibodies (primary antibodies must be from host species compatible to the secondary antibodies being used -- if using subclass specific antibodies, please refer to Technical Note “Western Blot and In-Cell Western™ Assay Detection Using IRDye® Subclass Specific Antibodies”).
- Tween® 20
- PBS Buffer (LI-COR P/N 928-40018 or 928-40020)
- Methanol (when using Immobilon®-FL PVDF membrane)
- SDS (when using Immobilon-FL PVDF membrane)
- Western Blot Incubation Box
 - Medium (8.9 x 6.6 x 2.9 cm), LI-COR P/Ns 929-97201 (1 pack), 929-97205 (5 pack), and 929-97210 (10 pack)

II. Gel Preparation for Blocker Optimization

Standard protein electrophoresis conditions and reagents can be used for gel and sample preparation. Following is a suggested template for sample electrophoresis to maximize blocker optimization and efficiently choose the best blocking conditions for a given primary antibody.

Using a 15-well gel, load the following samples in order indicated:

| Lane | Sample | Amount |
|------|------------------|---|
| 1 | Protein Marker | 2-10 μ L |
| 2 | Primary Antibody | 5 μ L of a 1:1000* dilution in PBS |
| 3 | Sample Lysate | 10 μ g |
| 4 | Sample Lysate | 5 μ g |
| 5 | Sample Lysate | 2.5 μ g |
| 6 | Protein Marker | 2-10 μ L |
| 7 | Primary Antibody | 5 μ L of a 1:1000* dilution in PBS |
| 8 | Sample Lysate | 10 μ g |
| 9 | Sample Lysate | 5 μ g |
| 10 | Sample Lysate | 2.5 μ g |
| 11 | Protein Marker | 2-10 μ L |
| 12 | Primary Antibody | 5 μ L of a 1:1000* dilution in PBS |
| 13 | Sample Lysate | 10 μ g |
| 14 | Sample Lysate | 5 μ g |
| 15 | Sample Lysate | 2.5 μ g |

* Suggested starting point; may need to be altered for concentration of primary antibody.

III. Western Blocker Optimization Method

Western blot should be prepared using standard blotting procedures and Millipore Immobilon®-FL PVDF or Odyssey Nitrocellulose Membrane. Allow blot to dry for at least 1 hour before proceeding with detection. Dry blots can be stored between filter paper overnight at room temperature, protected from light.

NOTE: Membranes should be handled only by their edges, with clean forceps. Take great care to never touch the membrane with bare or gloved hands.

NOTE: Do not write on membrane with an ink pen or marker, as the ink will fluoresce on the Odyssey Imager. Mark with pencil or Odyssey Pen (P/N 926-71804) to avoid this problem. Use pencil only for PVDF membrane, as wetting in methanol will cause ink to run.

If using the gel configuration outlined in the Gel Preparation for Blocker Optimization section above, cut the membrane, being careful not to touch the membrane along protein marker lanes 6 and 11 as shown in Figure 1. Label appropriately with pencil.

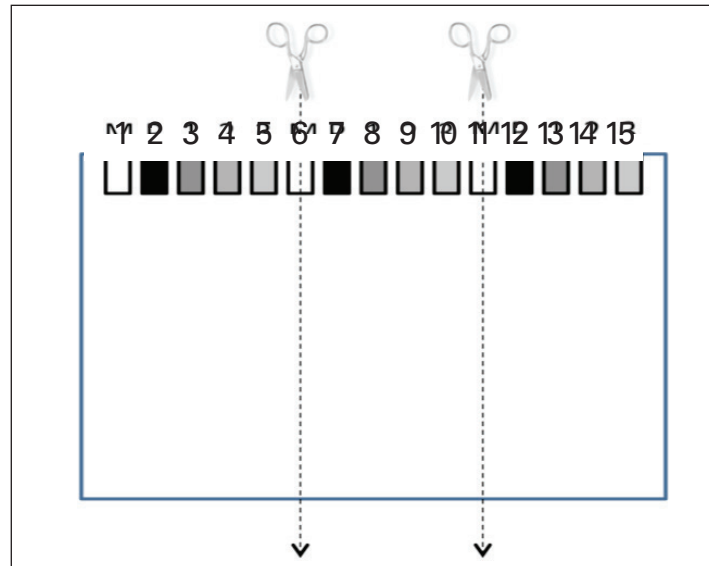


Figure 1. Cut Western blot along the Marker lanes into three individual optimization blots.

After cutting membrane, perform the following steps:

1. For Immobilon®-FL PVDF membranes:

- Pre-wet 1 minute in 100% methanol
- Rinse with ultrapure water
- Wet in 1X PBS for 2 minutes

For Odyssey Nitrocellulose Membranes:

- Wet in 1X PBS for 2 minutes

2. Place membranes into 3 different Western Blot Incubation Boxes and block the membrane in 10 mL Blocking Buffer for 1 hour while gently shaking.

- Box 1 – Odyssey Blocking Buffer
- Box 2 – Casein Blocking Buffer
- Box 3 – Blocking Buffer of your choice

3. Dilute primary antibody* in 10 mL of appropriate diluent listed below:

- Box 1 – Odyssey Blocking Buffer + 0.2% Tween® 20 + Primary Antibody
- Box 2 – Casein Blocking Buffer + 0.2% Tween 20 + Primary Antibody
- Box 3 – Blocking Buffer of your choice + Primary Antibody

* The correct working range for antibody dilution depends on the characteristics of your primary antibody. Start with the dilution recommended by the primary antibody vendor for Western blot applications.

4. Incubate blots in diluted primary antibody for 1 to 4 hours* at room temperature, or overnight at 4°C while gently shaking.

*incubation times vary for different primary antibodies

5. Wash membranes:
 - Pour off primary antibody solution.
 - Rinse membrane with 1X PBS-T (0.1% Tween® 20).
 - Cover blot with 1X PBS-T (0.1% Tween 20).
 - Shake vigorously on platform shaker at room temperature for 5 minutes.
 - Pour off wash solution.
 - Repeat 3 additional times.

6. Dilute secondary antibody* in 10 mL of appropriate diluent listed below:

Secondary antibody diluent for Immobilon®-FL PVDF membrane

 - Box 1 – Odyssey Blocking Buffer + 0.2% Tween 20 + 0.01% SDS + Secondary Antibody
 - Box 2 – Casein Blocking Buffer + 0.2% Tween 20 + 0.01% SDS + Secondary Antibody
 - Box 3 – Blocking Buffer of your choice + Secondary Antibody

Secondary antibody diluent for Odyssey Nitrocellulose Membrane

 - Box 1 – Odyssey Blocking Buffer + 0.2% Tween 20 + Secondary Antibody
 - Box 2 – Casein Blocking Buffer + 0.2% Tween 20 + Secondary Antibody
 - Box 3 – Blocking Buffer of your choice + Secondary Antibody

**For IRDye 800CW and IRDye 680RD conjugates, suggested dilution range is 1:5,000 to 1:25,000 and may require optimization. For IRDye 680LT conjugates, suggested dilution range is 1:20,000 to 1:50,000. Please consult pack insert.*

7. Incubate blot in diluted secondary antibody for 30-60 minutes at room temperature with gentle shaking.

Protect membrane from light during incubation.

8. **Protect from light during washes.**

Wash membranes:

 - Pour off secondary antibody solution.
 - Rinse membrane with 1X PBS-T (0.1% Tween 20).
 - Cover blot with 1X PBS-T (0.1% Tween 20) using same volumes indicated above for Western blot incubation boxes.
 - Shake vigorously on platform shaker at room temperature for 5 minutes.
 - Pour off wash solution.
 - Repeat 3 additional times.

9. Rinse membrane with 1X PBS to remove residual Tween 20. The membrane can be imaged wet or dry.

10. Image all three blots side-by-side.

11. Visual inspection or data analysis with Odyssey application or Image Studio software can be used to determine which blocking buffer works best with the evaluated primary.

Tips

- Follow the protocol carefully.
- For additional Odyssey Western detection tips, see www.licor.com/bio

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