Product Number
925-34015

Storage: 4 °C

Revised: February 7, 2017

Doc # 988-16578

4647 Superior Street • P.O. Box 4000
Lincoln, Nebraska 68504 USA
North America: 800-645-4267
International: 402-467-0700
FAX: 402-467-0819

LI-COR GmbH Germany, Serving Europe, Middle East, and Africa: +49 (0) 6172 17 17 771
LI-COR UK Ltd. UK, Serving UK, Ireland, and Scandinavia: +44 (0) 1223 422104
All other countries, contact LI-COR Biosciences or a local LI-COR distributor;
http://www.licor.com/distributors

www.licor.com/bio

Odyssey® Western Blotting Starter Kit 4

Kit Components

926-68170	IRDye® 680RD Goat anti-Mouse Secondary Antibody, 25 µL, 1 mg/mL
827-08365	IRDye 800CW Goat anti-Rabbit Secondary Antibody, 25 µL, 1 mg/mL
927-50100	Odyssey Blocking Buffer (TBS), 125 mL
826-14331	Odyssey Nitrocellulose Membranes, four – 10 x 10 cm

Quick Start Guidelines for New User Western Blot Preparation

If you have questions please contact your LI-COR Sales and Support Scientist, or call the technical support line, 1-800-645-4260.

1. Blots can be prepared and stored in advance.
2. Prepare replicate blots when comparing established methods:
    a. One following the LI-COR Protocol for Odyssey detection;
    b. One following your current detection method;
    c. Optional: prepare a blot using current methods and reagents but detect with LI-COR-supplied secondary antibodies.
3. Standard pre-stained blue markers can be visualized on the Odyssey Imager. Due to the sensitivity of the Odyssey Imager, load only 25% of the normal volume. It is best if the markers are barely visible on the gel; otherwise, the markers will be too strong on the Odyssey image.
4. ***Do not write on blot with a pen or Sharpie® marker***; use pencil only. Handle blot with clean forceps only.
5. For 1-color blots, use IRDye 800CW secondary antibody for detection of the protein.
6. For 2-color blots,
    a. Make sure primary antibodies are from different species (Example: Rabbit Polyclonal and Mouse Monoclonal)
    b. Use the IRDye 800CW secondary antibody to detect the low abundant protein and IRDye 680RD secondary antibody to detect the more abundant protein.
7. When processing Western blots, do not use any dishes that have been used for Coomassie staining. The Odyssey Imager is very sensitive to Coomassie and dishes used for staining will add a tremendous amount of background in the 700 channel.
8. ***Do not add Tween® 20 to Odyssey Blocking Buffer during the blocking step.***
9. Your normal primary antibody dilutions should be added to the Odyssey Blocking Buffer for primary incubation. Add 0.2% Tween 20 to the final concentration. Incubate the usual amount of time. ***Do not reduce the concentration of your primary antibody.***
10. ***Dilute secondary antibodies 1:20,000 in Odyssey Blocking Buffer.*** Add 0.2% Tween 20 to the final concentration at this point. If you are using PVDF, add 0.01% SDS to the secondary incubation. ***Do not add SDS if using nitrocellulose membrane.***
11. Incubate with secondary antibodies in the dark for one hour with gentle shaking. This can be done by covering the box with aluminum foil.
12. After the blot has been processed, store wet in 1X TBS for a maximum of 48 hours, or store dry blots at room temperature indefinitely. Keep all blots (wet or dry) in the dark until you are ready to image.

Detailed Western Blot Procedure

Western blot should be prepared using standard blotting procedures using the nitrocellulose provided in this kit or of choice. Allow transferred blot to dry for at least 1 hour before proceeding with detection. Dry blots can be stored at room temperature between filter paper overnight.

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 they will fluoresce on the Odyssey® Imager. Mark with pencil to avoid this problem.

Perform the following steps:

1. For Immobilon-FL PVDF membrane:
    a. Pre-wet 1 minute in 100% methanol.
    b. Rinse with ultra pure water.
    c. Wet in 1X TBS for 2 minutes.

    For nitrocellulose membrane (provided in this kit):

    a. Wet in 1X TBS for 2 minutes, or until fully hydrated.

2. Place membrane in incubation box and block the membrane in Odyssey Blocking Buffer for 1 hour with gentle shaking.

3. Prepare primary antibody dilution:
    a. Prepare the primary antibody diluent. Add Tween® 20 to Odyssey Blocking Buffer for final concentration of 0.2% Tween 20.
    b. Dilute primary antibody in Odyssey Blocking Buffer with 0.2% Tween 20 using the vendor's recommended dilution for Western blot applications for that primary antibody. Dilutions may range from 1:200 -1:5000, depending on the primary antibody.

4. Incubate blot in diluted primary antibody for 1 to 4 hours* at room temperature, or overnight at 4 °C with gentle shaking.
    **Optimal incubation times vary for different primary antibodies.*

    

    If the procedure cannot be completed in full, this is a good place to stop until the following day by incubating the primary antibody overnight at 4 °C with gentle shaking.

5. Wash membranes:
    - Pour off primary antibody solution.
    - Rinse membrane with 1X TBS-T (0.1% Tween 20).
    - Cover blot with 1X TBS-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 provided in Western Kit 1:20,000*** *in the appropriate diluent listed below:*
    a. ***Secondary antibody diluent for nitrocellulose membrane***
    - Add Tween 20 to a final concentration of 0.2% in Odyssey Blocking Buffer

    b. ***Secondary antibody diluent for Immobilon®-FL PVDF membrane***
    - Add Tween 20 to a final concentration of 0.2% **and** SDS to a final concentration of 0.01% in Odyssey Blocking Buffer.

7. Protect membrane from light during incubation. Incubate blot in diluted secondary antibody for one hour at room temperature with gentle shaking.

8. ***Protect Membrane from light during washes****.*
    Wash membranes:
    - Pour off secondary antibody solution.
    - Rinse membrane with 1X TBS-T (0.1% Tween® 20).
    - Cover blot with 1X TBS-T (0.1% Tween 20).
    - Shake vigorously on platform shaker at room temperature for 5 minutes.
    - Pour off wash solution.
    - Repeat 3 additional times.

9. Rinse membrane with 1X TBS to remove residual Tween 20. The membrane can be stored in 1X TBS for 48 hours in the dark at 4 °C until you are ready to image.

Alternatively, blots can be prepared more than 48 hours in advance. Dry the blot and store in the dark at room temperature until ready to image.

Optimization Tips

- ***Follow the protocol carefully.***
- No single blocking reagent will be optimal for every antigen-antibody pair. Some primary antibodies may exhibit greatly reduced signal or different non-specific binding in different blocking solutions. If you have difficulty detecting your target protein, changing the blocking solution may dramatically improve performance. If the primary antibody has worked well in the past using chemiluminescent detection, try that same blocking solution for Odyssey detection.
- To avoid background speckles on blots, use high-quality ultra pure water for buffers. Rinsing previously used incubation boxes with methanol can reduce background contamination of future blots.
- Never perform Western incubations or washes in dishes that have been used for Coomassie staining.
- Membranes should be handled only by their edges, with forceps.
- Always pour off antibody solution and washes from the same corner of the box to ensure complete removal of previous solutions.
- After handling membranes that have been incubating in antibody solutions, clean forceps thoroughly with distilled water and/or methanol, then rinse with distilled water.
- Do not wrap the membrane in plastic when scanning.

Guidelines for Two-Color Western Blot Detection

Two different antigens can be detected simultaneously on the same blot using IRDye® secondary antibodies. When performing a two-color blot, use the standard Western blot protocol with the following modifications:

- Combine the two primary antibodies in the diluted antibody solution in step 3. Incubate simultaneously with membrane (step 4). The primary antibodies must be from two different host species.
- Combine the two IRDye secondary antibodies in the diluted antibody solution in step 6. Incubate simultaneously with membrane (step 7).

Two-color detection requires careful selection of primary and secondary antibodies. The following guidelines provide further information that will help you successfully design two-color experiments:

- The two primary antibodies must be derived from different host species so that they can be discriminated by secondary antibodies of different specificities (for example, primary antibodies from rabbit and mouse will be discriminated by anti-rabbit and anti-mouse secondary antibodies, respectively).
- One secondary antibody must be labeled with IRDye 680RD and the other with IRDye 800CW.
- In general, it is recommended that the IRDye 800CW secondary antibody be used to detect the low abundant protein and IRDye 680RD secondary antibody to detect the more abundant protein.
- Always use highly cross-adsorbed secondary antibodies for two-color detection. Failure to use cross-adsorbed antibodies may result in increased cross-reactivity of the secondary antibodies. The antibodies in this kit have been optimized for two-color detection.
- For best results, avoid using primary antibodies from mouse and rat together in a two-color experiment. The two species are so closely related it is not possible to completely adsorb away all cross-reactivity. If there is no other option but to use mouse and rat together, it is crucial to run single-color blots first with each individual antibody to be certain of expected band sizes.

Full Size Reagent Reorder Information

IRDye® 800CW Goat anti-Rabbit IgG, 0.5 mg	P/N 926-32211
IRDye 680RD Goat anti-Mouse IgG, 0.5 mg	P/N 926-68070
Odyssey® Blocking Buffer (TBS), 500 mL	P/N 927-50000
Odyssey Nitrocellulose Membranes, 1 Roll	P/N 926-31092

These products are offered for research and development purposes only. They cannot be used to manufacture derivatives or products for resale.