Aug 24, 2020

Western Blot Detection using Licor NIR Fluorescence - CHEM 584

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In Development dx.doi.org/10.17504/protocols.io.bj9ckr2w

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Adapted from Licor manufacturer instructions.

DOI

dx.doi.org/10.17504/protocols.io.bj9ckr2w

PROTOCOL CITATION

Ken Christensen 2020. Western Blot Detection using Licor NIR Fluorescence - CHEM 584 . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj9ckr2w

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Adapted from Licor manufacturer instructions.

CREATED

Aug 24, 2020

LAST MODIFIED

Aug 24, 2020

PROTOCOL INTEGER ID

40964

GUIDELINES

Quick Start Hints and Tips for Western Blot Detection

1. Store the IRDye secondary antibody vial in darkness at 4°C. Minimize exposure to light and take care not to introduce contamination into the vial. Dilute immediately prior to use. If particulates are seen in the antibody solution, centrifuge before use.

2. The best transfer conditions, membrane, and blocking agent for experiments will vary, depending on the antigen and antibody.

3. Do not write on blot with a pen or Sharpie® marker. Ink from most pens and markers will fluoresce in the 700 nm channel of all Odyssey® Family Imaging Systems. The ink may wash off and re-deposit elsewhere on the membrane, causing increased background. Use a pencil to mark membranes.

4. Handle blot with clean forceps only.

5. Before using forceps, incubation trays, and the Odyssey scanning surface or sample tray (if applicable), clean with 100% methanol to remove any residual dye signal from previous use. Rinse with a small volume of distilled water, followed by isopropanol. Dry with a lintfree wipe.

6. When processing Western blots, do not use dishes/boxes that have ever been used for Coomassie staining. The Odyssey imagers are very sensitive to Coomassie (which is a strongly-fluorescent dye), and use of dishes with small traces of Coomassie will add a tremendous amount of background in the 700 nm channel. Maintain the same buffer system throughout the Western blot process. For example, if you block your blot in Odyssey Blocking Buffer (PBS), use PBS-based buffers throughout the protocol.

7. Do not include detergents during the blocking step.

8. Incubate with secondary antibodies in the dark for one hour with gentle shaking. The incubation box can be covered with aluminum foil.

After the transfer, complete the following steps

1 Wet in 1x PBS or TBS for **00:02:00** , or until fully hydrated (using the appropriate buffer system).

2 Place membrane in incubation box and block the membrane in 5% milk (PBS or TBS +0.2% v/v Tween® 20) for **00:15:00** with gentle shaking. Be sure to use a sufficient blocking buffer to cover the membrane (a minimum of 0.4 mL/cm^2 is suggested).

3 Prepare primary antibody:

a. Dilute primary antibody in Odyssey Blocking Buffer + 0.2% Tween 20, using the vendor's recommended dilution for Western blot applications. Dilutions typically range from 1:200 - 1:5,000, depending on the primary antibody.

b.Use enough antibody solution to completely cover the membrane. Put the membrane in a small ziplock baggy, this will allow a small volume of antibody to effectively cover the membrane area.

4 Incubate blot in diluted primary antibody for **01:00:00** to **04:00:00** * at room temperature with gentle shaking, or overnight at 4 °C.

If the procedure cannot be completed in full, this is a good place to stop until the following day. Incubate the blot in primary antibody overnight at 4°C with gentle shaking, and resume the protocol the next day.

**NOTE: Optimal incubation times vary for different primary antibodies.*

5 After the primary antibody incubation, wash membranes:

a. Pour off the primary antibody solution.

b. Rinse membrane with appropriate buffer – 1x TBS-T (0.1% Tween® 20) or 1x PBS-T (0.1% Tween 20).

c. Cover blot with 1x TBS-T (0.1% Tween 20) or 1x PBS-T (0.1% Tween 20).

d. Shake vigorously on a platform shaker at room temperature for **00:05:00** .

e. Pour off wash solution.

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f. Repeat additional times.

b. Rinse membrane with appropriate buffer – 1x TBS-T (0.1% Tween® 20) or 1x PBS-T (0.1% Tween 20). Do not add SDS.

NOTE: A Suggested dilution range for secondary antibodies is typically 1:5,000 to 1:25,000. Recommended dilutions can be found on the secondary antibody pack insert. Use 1:20,000 as a suggested starting point.

6 Protect the membrane from light during incubation. Incubate blot in diluted secondary antibody for **01:00:00** at room temperature with gentle shaking. Protect the membrane from light during washes.

7 Wash membranes:

a. Pour off secondary antibody solution.

b. Rinse membrane with the appropriate buffer – 1x TBS-T (0.1% Tween 20) or 1x PBS-T (0.1% Tween 20).

c. Cover blot with 1x TBS-T (0.1% Tween 20) or 1x PBS-T (0.1% Tween 20).

d. Shake vigorously on platform shaker at room temperature for **00:05:00** .

e. Pour off wash solution.

f. Repeat additional times.

8 Rinse membrane with 1x TBS/PBS (as appropriate) to remove residual Tween 20.

9 The Western blot is now ready to image. The membrane can be stored in 1x TBS or 1x PBS for up to 48 hours in the dark at 4°C. If the blot is prepared more than an hour in advance, air-dry the blot and store in the dark at room temperature until ready to image.

10 10. Image with an Odyssey® Family Imaging System. If the signal on the membrane is too strong or too weak, adjust the imaging parameters to optimize the image.

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