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 We are still developing and optimizing this protocol

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Fluorescent Western Protocol V.1

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ABSTRACT

Analysis of proteins using fluorescent immunoblot.

Note:

- The choice of secondary antibody depends on the choice of primary antibody, whether it is derived from a mouse (monoclonal) or a rabbit (polyclonal).
- It is advisable to stick to the 800CW wavelength to avoid problems with chlorophyll autofluorescence encountered with the 680CW antibodies.

Literature: <https://www.licor.com/documents/fxc6evvxbub4srkqy6i9yg46l7i0xz5>

MATERIALS

- Intercept^R PBS Blocking Buffer (LI-COR Biosciences; [927-70001](#)) - milk powder with PBS could equally be used
- IRDye[®] 800CW Donkey anti-Rabbit IgG Secondary Antibody (LI-COR Biosciences; [926-32213](#))
- IRDye[®] 800CW Donkey anti-Mouse IgG Secondary Antibody (LI-COR Biosciences; [926-32212](#))
- Black Western Blot Incubation Box (LI-COR Biosciences; [929-97110](#))
- 10x PBS buffer
- TweenTM 20 (Fisher Biosciences; [BP337-100](#))
- Methanol
- Odyssey CLx Imager (LI-COR Biosciences)
- Primary antibody ([various](#))

1

Keep membranes in the black Western Blot incubation box for all steps, this is important after

adding the secondary antibody because the signal is light-sensitive and will become bleached if not exposed to light for a long enough period.

2 Wet with 1x PBS for 00:02:00 min 2m

3 Rinse membrane with dH₂O

4 Discard PBS and incubate with Intercept (PBS) Blocking Buffer **LI-COR Catalog #927-70001** 01:00:00 at Room temperature 1h

Note

As an alternative to Intercept Blocking Buffer you can use PBS-T 5% w/v milk powder. This is to prevent unspecific binding of antibody and lowers background signal

5 Incubate with primary antibody (appropriate dilution in Intercept Blocking Buffer) at 4 °C Overnight (in cold room) with gentle agitation on a platform shaker 50 rpm, 4°C .

6 Prepare 1 L PBS-T solution

7 Pour off the primary antibody and rinse the membrane with PBS-T.

Note

Some primary antibodies can be re-used multiple times depending on the concentration used, in this instance collect the primary antibody in a tube and store the solution at -20 °C before re-use.

- 8 Cover the membrane with PBS-T, shake vigorously on a platform shaker at 50 rpm, Room temperature , 00:10:00 . Repeat 3 times

- 9 Create a working dilution of secondary antibody using Intercept (PBS) Blocking Buffer **LI-COR Catalog #927-70001** with 0.2 % (v/v) Tween, 0.01 % SDS (w/v). IRE Dye secondary antibody (1:20,000).

Note

10 mL is sufficient to cover one blot and 0.01% SDS is only to be used in conjunction with PVDF membranes.

- 10 Incubate for 01:00:00 Room temperature with gentle agitation on a platform shaker. 1h

- 11 Pour off the secondary antibody

- 12 Cover the membrane with PBS-T, agitate 80 rpm, Room temperature , 00:10:00

- 13 Discard PBS-T. Repeat step 12 three times go to step #12

Note

Note: more washes (x5) and for longer can be done to reduce background

14 Rinse then cover the membrane with 1x PBS, 80 rpm, Room temperature , 00:10:00 . Repeat (2X)

15 Proceed to imaging blot on LI-COR Odyssey CLx imaging system

Equipment

Odyssey CLx	NAME
Imaging System	TYPE
LI-COR	BRAND
Odyssey CLx	SKU
https://www.licor.com/bio/odyssey-clx/	LINK