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Fluorescent Western Protocol

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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/fxc6evxvxbub4srkqy6i9yg46l7i0xz5

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MATERIALS TEXT

- 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
- Tween^{TM} 20 (Fisher Biosciences; BP337-100)
- Methanol
- Odyssey CLx Imager (LI-COR Biosciences)
- Primary antibody (various)

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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 dH_2O

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

at **Room temperature**

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.

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 ^{o}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).

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: 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

Odyssey CLx
Imaging System

LI-COR **Odyssey CLx**