

abcam

Fluorescent western blot

Protocol for IRDye[®] secondary antibodies

Introduction

Fluorescent western blot is more quantitative and provides wider dynamic range than enzyme-based approaches, making it a good option for quantifying relative protein abundance.

This protocol covers membrane blocking, probing with antibody, and imaging steps only. For guidance on sample preparation, running gels, and transferring protein from gel to membrane, [see our general western blot protocol](#).

Procedure

Membrane blocking and probing with primary antibody

1. Place membranes in Petri dishes and cover with high-quality TBS-based blocking buffer (BB). Incubate for 1 h.
2. Dilute antibody in dilution buffer (50 % BB, 50% TBS 0.1% Tween 20 (TBST)).
3. Incubate membranes with 10 mL of the primary antibody solution on a rocking platform. Optimal dilution and incubation times should be determined using serial dilutions and a time course.

Washing the membranes and preparing the secondary antibody

1. Discard the primary solution and begin washing steps, keeping the membrane in the original Petri dish/wash tray.
2. Wash the membrane with TBST using 2 brief rinses, 5 mL each, followed by enough buffer to cover the membrane for 1 x 15-minute wash and 3 subsequent 10 minute washes at room temperature.

Place the wash trays and petri dishes on a rocker during the washes ensuring that the blots are washed separately.

3. Between washes, dilute the secondary antibodies in antibody dilution buffer according to the WB experimental plan (see Table 2 below). Ensure that the secondary antibody is thoroughly mixed with the dilution buffer.

All secondary antibodies are light sensitive and should be covered during incubation.

4. Discard the TBST and add the required amount of secondary antibody. Incubate the membrane with the corresponding secondary on the rocker at room temperature for 1 hour protected from light.
5. After incubation, discard the secondary solution and repeat washing procedure in Step 2, keeping the membrane in the original Petri dish or wash tray

Table 1. Secondary antibodies and dilutions

Species that primary antibody was raised in	Required secondary antibody	Recommended dilution
Rabbit	Goat anti-rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773)	1:10,000
	Goat anti-rabbit IGG H&L (IRDye® 680RD, ab216777)	1:10,000
Mouse	Goat anti-mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772)	1:10,000
	Goat anti-mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776)	1:10,000

Imaging

1. Clean the glass scanning bed with 70% ethanol using a Koton lint-free cloth.
2. If scanning the membranes dry, place them between two sheets of filter paper and cover with foil and leave on the bench overnight. Place the dry membranes protein side down onto the glass scanning bed the silicon mat on top of the membrane to ensure the membranes lie flat against the glass. There is no need to roll.
3. If scanning the membranes wet, place the membranes on the scanning bed protein side down and spray 5 mL of TBST across the scan area. Place a silicon mat on the membranes and use a roller to remove any bubbles.

Scanning the membranes wet works well with histones.

4. Close the equipment lid. Scan strips accordingly to equipment instructions. Remove the membranes from the scan bed and clean the scan bed with 70% ethanol using a lint-free cloth.