Guidelines for Preparing Demo Western Blots for the ODYSSEY® Infrared Imaging System

Prepare replicate Western Blots for comparison – at least one for imaging on the Odyssey Imager, and one for your current method. Feel free to also run a Coomassie gel to test the sensitivity of Coomassie on the Odyssey Imager.

Below are some guidelines for preparing the Western Blots for imaging on the Odyssey, which we kindly ask you to follow.

**Electrophoresis step:**

- Load a molecular weight marker on BOTH sides of the gel if needing to determine MW sizing. Preferably use LI-COR Two-Color Protein Marker. Blue-stained MW markers are also visible on the Odyssey (in the 700 channel). If you use such, load only about 25% of the amount typically loaded.
- Run the loading dye front out off the gel to reduce background at lower molecular weight sizes.

**Membrane choice and handling:**

- Feel free to use any brand of nitrocellulose if that is what you normally use. If using PVDF, be sure to use the PVDF provided by LI-COR (Millipore Immobilon-FL), as other brands give increased background signal.
- Use only pencil or the LI-COR Odyssey Pen to mark membranes. Do not use pens, as these give background fluorescence.
- Handle membranes carefully and with forceps (fingerprints will show up on the Odyssey).
- When processing Western Blots, do not use any dishes that may have been used for Coomassie staining previously. The Odyssey Imager is very sensitive for Coomassie, and Coomassie residue in dishes will add high background fluorescence.

**Membrane blocking:**

- Use LI-COR Odyssey Blocking Buffer for blocking the membrane.
- Do NOT add Tween® 20 or SDS to the Blocking Buffer, as this will lead to high background fluorescence.

**Antibody considerations and handling:**

- All antibody incubations should be performed in LI-COR’s Odyssey Blocking Buffer.
- Add Tween® 20 to antibody dilutions at a concentration of 0.1% (further optimization can occur subsequently).

  **Primary antibodies:**
  - Usual primary dilution ratio should be used for primary incubation. Incubate the usual amount of time.
  - Do NOT add SDS to the primary antibody.
  - For two-color detection, primary antibodies raised in different host species must be used, e.g. mouse and rabbit; unless using LI-COR IRDye Goat anti-Mouse IgG subclass-specific secondary antibodies.

  **Secondary antibodies:**
  - Dilute the secondary antibodies **1:10,000-20,000**. Incubate the membrane for 1 hour at room temperature.
  - For 2-color blots, use IRDye 800CW 2° AB to detect low abundant protein and IRDye 680LT to detect the more abundant protein.
  - If using PVDF membranes, add SDS (final concentration of 0.02%) and Tween® 20 (final concentration of 0.1%) to the secondary antibody dilution to avoid non-specific background staining.
  - Fluorophore labeled antibodies are light–sensitive. Use LI-COR’s Western incubation boxes or cover your box with foil during incubation.

After the final washing step, blots may be stored in PBS at 4 °C though it is not recommended to do so for longer than 48 hrs. prior to imaging. Blots may also be stored dry for a period of months between blotting paper. In either case, protect from light and do not wrap membranes in plastic wrap.