ODYSSEY®
Quick**Card**

Western Blots

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Sample Protocol

1. Wet membrane in 1X PBS/TBS* for 5 minutes. (Pre-wet PVDF membrane in 100% methanol and rinse with ultra-pure water before wetting.)
2. Block membrane with Intercept® Blocking Buffer for 1 hour at room temperature with gentle shaking.
3. Decant blocking buffer.
4. Add primary antibody using the vendor's recommended dilution for Western blot applications in Intercept T20 Antibody Diluent**. Incubate a minimum of 1 hour at room temperature with gentle shaking.
5. Wash membrane 4 times for 5 minutes in a generous amount of PBS/TBS* plus 0.1% Tween® 20 with gentle shaking.
6. Dilute the IRDye® secondary antibody (consult the IRDye secondary antibody pack insert) in Intercept T20 Antibody Diluent** (also add 0.01% - 0.02% SDS for PVDF) and incubate, protected from light, 1 hour with gentle shaking.
7. Wash membrane as in step 5; protect from light.
8. Rinse membrane in 1X PBS/TBS*.
9. Scan on an Odyssey Imaging System.

Two-Color Detection

- In step 4, combine two primary antibodies of different species and incubate simultaneously with the membrane.
- In step 6, combine the IRDye 680RD and IRDye 800CW secondary antibodies that recognize the primary antibodies and incubate simultaneously with membrane.

For Best Results

- Let membrane dry after transfer for 1 hour or overnight.
- Clean forceps, incubation trays, and Odyssey scanning surface (if applicable) before use 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 lint-free wipe.
- Handle membranes by the edges only. Be sure to use clean forceps with smooth edges.
- Avoid pouring antibody or wash solutions directly onto membranes; pour onto the bottom of the dish or down the side.
- Do not include detergents during the blocking step.

** Maintain the same buffer system (PBS or TBS) throughout the Western blot process.*

*** If using an alternative blocking buffer, add 0.2% Tween 20 to the primary and secondary antibody dilutions.*

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