WESTERN BLOT ANALYSIS USING THE ODYSSEY INFRAFED IMAGING SYSTEM

The Odyssey system incorporates fluorescently-labeled antibodies that can be detected by scanning at the appropriate wavelength. The resulting scan can be analyzed for quantitation or band sizing. The user manual gives in-depth instructions for the software applications and data manipulation.

Reagents:
IR-labeled secondary antibodies
Blotted nitrocellulose or PVDF membrane
Primary antibody
1X PBS/Tween-20 (0.1-0.5%)

Procedure:

1. Transfer protein to membrane by standard procedure and rinse briefly in 1X PBS.
2. Block the membrane in PBS/5% NFDM for one hour. **DO NOT** add Tween-20 or BSA when blocking the membrane or high background will result.
3. Wash 3x 5 minutes with 1X PBST with gentle shaking, using a generous amount of buffer.
4. Incubate blot one hour (or optimum incubation time) in primary antibody diluted in PBST. **NEVER** use BSA or BSA containing solutions for antibody dilution or high background will result.
5. Wash membrane 3x 5 minutes in PBST with gentle shaking, using a generous amount of buffer.
6. Dilute the fluorescently-labeled secondary antibody in PBST/0.5-1.0% NFDM. Avoid prolonged exposure of antibody vial to light. Suggested dilution range is 1:2000-1:10,000.
7. Incubate blot in secondary antibody for 60 minutes with gentle shaking. Protect from light during the incubation. Allowing incubation to proceed more than 60 minutes may increase background.
8. Wash membrane 3x 5 minutes in PBST.
9. Rinse membrane in PBS to remove residual Tween. The membrane is now ready to scan. The membrane may be kept wet or dried prior to scanning; once a membrane has dried, stripping will be ineffective.

Comments:

- Membranes can be blocked overnight at 4°C.
- Diluted secondary antibody can be saved and reused if it is not stored in a milk-based buffer. Store at 4°C and protect from light.
- The fluorescent signal on the membrane will remain stable for several weeks or longer if protected from light. Membranes may be stored dry or in PBS buffer at 4°C.
- Signal strength may be enhanced on a dry membrane.

- Higher background may result when using PVDF membranes.

Reference:
Odyssey Infrared Imaging System User Guide and Protocols at
biosupport.licor.com/support

Submitted by:
Jennifer Suddoth