Adapting Membrane Antibody Array Protocol for Odyssey® System

Abcam membrane antibody arrays (detecting cytokines and phosphorylated proteins) can be easily adapted for detection on the Odyssey® system, with a few minor modifications.

1. Required Reagents

Antibody array components used with Odyssey:
- Cytokine or phosphorylation membrane antibody arrays
- Biotin-Conjugated antibody
- 20X Wash Buffer I
- 20X Wash Buffer II
- 2X Cell Lysis Buffer
- Eight-Well Incubation Tray
- Manual

Components not used with Odyssey:
- 2X Blocking Buffer
- Detection Buffer A, B, C, or D
- 20,000X HRP-Conjugated Streptavidin

Additional Reagents Required
- Odyssey Blocking Buffer (LI-COR, Cat. #927-40000)
- Tween®-20.
- IRDye® 800CW-streptavidin (LI-COR, Cat. #926-32230); or IRDye® 680-streptavidin (LI-COR, Cat. #926-32231)

2. Guidelines for adapting membrane arrays for detection on the Odyssey System

Use **Odyssey Blocking Buffer** supplemented with Tween-20 (0.05% final concentration) **instead of** the blocking buffer referenced provided.
- Just before use, prepare blocking buffer by adding 50 µL of 20% Tween-20 to 20 mL of Odyssey Blocking Buffer. If desired, larger amounts of this solution may be prepared and stored at 4 °C for up to one month.

Use **IRDye-labeled streptavidin instead of** the HRP-conjugated streptavidin provided.
- Prepare a 1:2000 dilution of streptavidin conjugate immediately prior to use by adding 1 µL IRDye-labeled streptavidin to 2 mL of blocking solution (Odyssey Blocking Buffer + 0.05% Tween-20); mix well by inversion. Optimal streptavidin concentration may require experimental determination.

After IRDye-labeled streptavidin incubation, each membrane should be washed at least four times in 2 mL Wash Buffer I and two times in 2 mL Wash Buffer II to ensure complete removal of extraneous dye on the membrane surface. For each wash, incubate membrane(s) for 5 minutes with gentle shaking or rocking.

After IRDye-labeled streptavidin detection and the subsequent washes, proceed directly to imaging on the Odyssey System.

Membrane antibody arrays should not be allowed to dry at any time prior to scanning. Membranes should be scanned wet and care should be taken to avoid bubbles when placing them on the Odyssey scanning surface.
- Store membranes in Wash Buffer II to prevent drying.

Odyssey scan parameters are:
- Resolution: 84 µm
- Quality: Medium
- Focus offset: 0.0 mm
- Intensity: Begin with intensity 5 (either channel, as appropriate) and adjust as needed, ensuring that there are no saturated (white) pixels in the spots of interest.

3. Membrane antibody array protocol adjusted for use with the Odyssey System

The following procedure should be considered only as a general guide when using Abcam membrane antibody arrays for detection on the Odyssey System.

All incubations may be performed at room temperature (unless otherwise noted) with gentle shaking or rocking. Carefully decant solutions and buffers using a pipet or vacuum aspirator.

Prepare samples as described in the protocol booklet.

Place each membrane into the provided eight-well tray

Add 2 mL of blocking solution (**Odyssey Blocking Buffer** with 0.05% Tween-20) to each membrane and incubate for 1 hour.

Decant the blocking solution and add 1 mL of the appropriate sample to each membrane in the tray (dilute with blocking solution, if necessary).
Incubate for 2 hours. Alternatively, incubate at 4 °C overnight.

Decant sample(s) and wash 3 times with 2 mL of 1X Wash Buffer I.
Wash 2 times with 2 mL of 1X Wash Buffer II. Allow 5 minutes for each wash.

Prepare biotin-conjugated antibody.
Add 100 µL of blocking solution to the antibody tube provided with the kit and mix gently.
Centrifuge tube briefly and transfer the mixture to a tube containing 2 mL of blocking solution.
Mix gently.

Add 1 mL of diluted biotin-conjugated antibodies to each membrane
Incubate for 1 hour.

Decant antibody solution and wash 3 times with 2 mL of 1X Wash Buffer I.
Wash 2 times with 2 mL of 1X Wash Buffer II.
Allow 5 minutes for each wash.

Add 2 mL of **IRDye-labeled streptavidin** solution, diluted 1:2,000 in blocking solution, to each membrane.
Incubate for 45 minutes.

Do not allow membrane(s) to incubate in IRDye-labeled streptavidin solution for more than 1 hour, as higher background fluorescence may result.

Decant streptavidin solution and wash at least four times with 2 mL of 1X Wash Buffer I.
Wash 2 times with 2 mL of 1X Wash Buffer II.
To prevent drying, allow membrane(s) to remain in Wash Buffer II until ready to scan on the Odyssey.
Allow 5 minutes for each wash.

Scan on the Odyssey Imager, following the Guidelines in section 3.

Membranes can be stored for later analysis either wet or dry, and protected from light, at 4 °C if desired. Prior to re-scanning membranes, wet briefly in Wash Buffer II. Membranes scanned dry will exhibit higher signal, but also higher background fluorescence.

For best assay results, include a negative control in which the sample is replaced with an appropriate mock buffer, especially when working with serum-containing media.