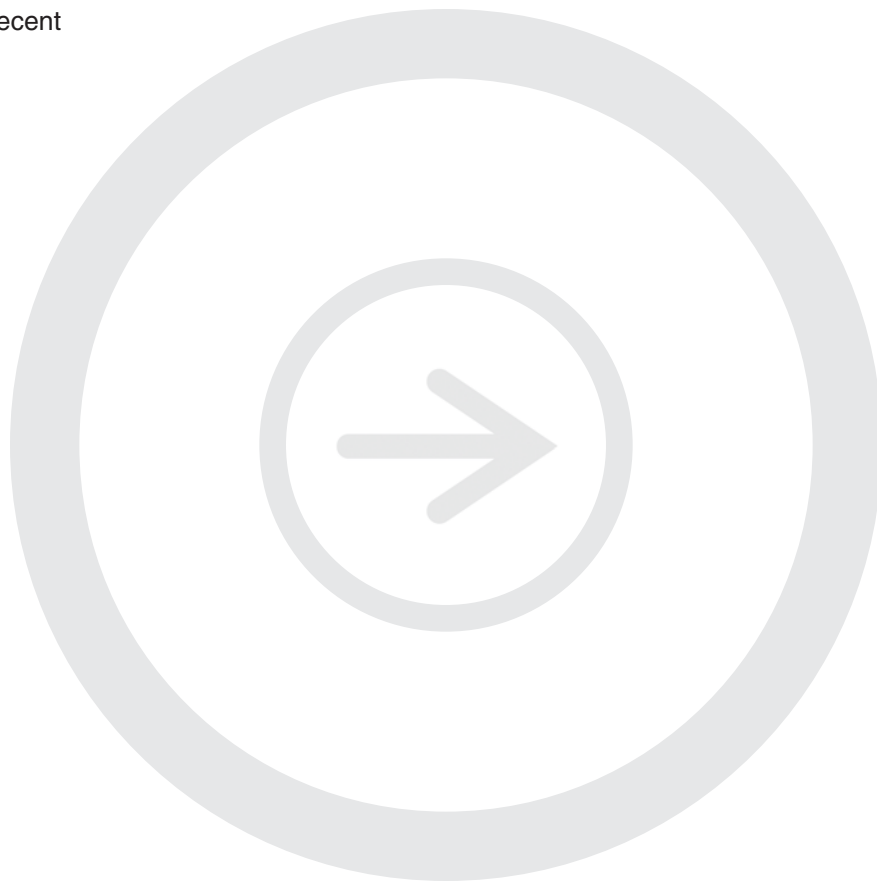


Odyssey[®]

Infrared Imaging System

Panorama[®] Mouse/Rat Tissue Extract Protein Array Detec- tion on the Odyssey[®] System

Revised January, 2007. The most recent
version of this protocol is posted at
<http://biosupport.licor.com/support>



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Doc# 988-08990

I. Introduction

The Panorama[®] Mouse/Rat Tissue Extract Protein Array (Sigma, Cat.# MRPA1) is a reverse phase protein array containing a panel of tissue extracts from mouse and rat. For each tissue, a total tissue, nuclear, and cytoplasmic extract is spotted in 3 concentrations. These arrays are designed to study protein expression in different tissues of mouse and rat simultaneously.

For detailed product information and availability please contact Sigma at 1-800-325-5832 or visit www.sigma-aldrich.com. For Odyssey support, contact LI-COR Biosciences at 1-800-645-4267.

II. Required Reagents

Panorama Mouse/Rat Extract Protein Array Kit Components

- Panorama Mouse/Rat Tissue Extract Protein Array Slides
- QuadriPERM[®] Cell Culture Vessel
- Tris Buffered Saline, pH 8.0, with Tween[®]-20
- Preincubation Buffer

Additional Reagents and Equipment Needed

- Protein specific primary antibody
- IRDye[®] 800CW labeled secondary antibody specific to the species in which the primary antibody was developed
- Odyssey Blocking Buffer (LI-COR Biosciences, Cat. # 927-40000) or appropriate blocking reagent for optimal antibody detection
- Tween[®]-20

III. Tips for Detection on the Odyssey System

Best results can be obtained by following the Panorama Mouse/Rat Tissue Extract Protein Array Kit Technical Bulletin (<http://www.sigmaaldrich.com/sigma/bulletin/mrpa1bul.pdf>), with the following modifications:

- Follow Steps 1-3.
- Step 4 - After aspiration of the preincubation buffer from the incubation tray and washing of the array, incubate the array in Odyssey blocking buffer *instead* of washing buffer for 30-40 minutes.
- Step 5 - Dilute primary antibody to appropriate concentration in 4 ml of Odyssey blocker. Invert tube to mix.
- Continue with Steps 6-9. Skip step 10.
- Step 11 - Dilute IRDye 800CW labeled antibody 1:4000 in 4 ml Odyssey blocking buffer with 0.01% Tween-20. Prepare blocking buffer by adding 25 µl of 20% Tween-20 to 50 ml of Odyssey Blocking Buffer; store at 4 °C.

NOTE: FROM THIS POINT ON KEEP ARRAY IN THE DARK TO PREVENT PHOTOBLEACHING! This can be done by covering the cell culture vessel with foil or a cardboard box.

- Step 12- Incubate for 1 - 2 hour at room temperature, gently shaking.
- Wash slide 3 X for 10 minutes in 4 ml of washing buffer.
- Let slide dry for at least 20 minutes before imaging. Drying by centrifugation is optimal.
- Image the array on the Odyssey, the following settings are recommended as a starting point and should be adjusted for optimal results:
 - Resolution: 42 μm
 - Focus offset: 0.0 mm
 - Intensity: 5 (800 channel)
- Quantify images using Odyssey Software or ArrayPro[®] Analyzer 4.5 Software (LI-COR, Part # 926-30050)

IV. Other Notes

The Sigma Panorama Mouse/Rat Tissue Extract Protein Array Technical Bulletin states that these arrays are semi-quantitative. Detection on the Odyssey system makes these arrays more quantitative; however, it is still highly recommended that results be further validated.

There are low levels of auto fluorescence of the tissue extracts in the 700 nm channel. For 1-color analysis, the 800 nm channel is optimal. Two-color analysis can be done by addition of a second primary antibody and IRDye secondary antibody for detection.

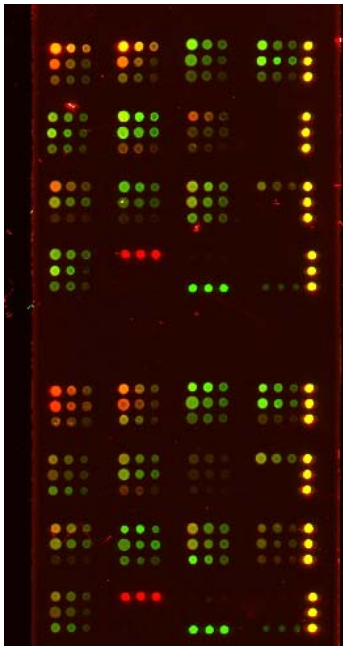


Figure 1. Sigma Panorama Mouse/Rat Tissue Extract Protein Array detected with Anti-Vinculin (800 nm Channel) and Anti- Tubulin (700 nm Channel).

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