

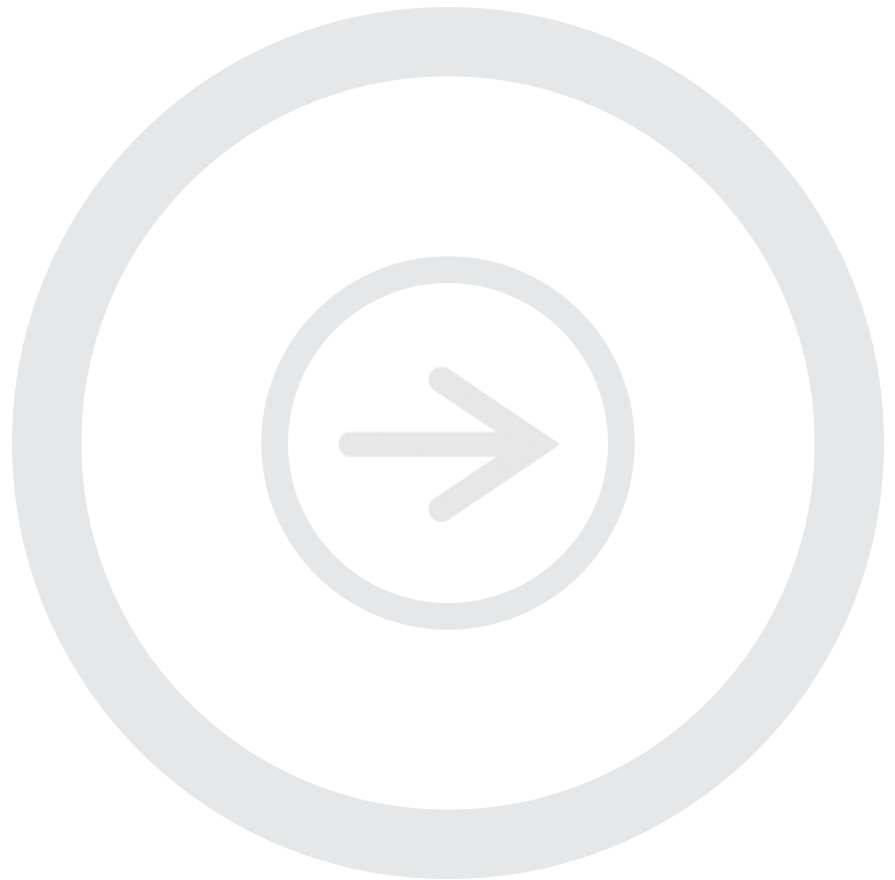
Odyssey[®]

Infrared Imaging System

Technical Note

Tips for Antibody and Lysate Arraying

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LI-COR[®]

Biosciences

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I. Tips For Antibody Arraying

See V&P Scientific, Inc. "Technical Note 42" for a detailed description of the care and use of the Manual Glass Slide Microarrayer.

Antibody Arraying

1. Getting Started

- Antibody arraying can be done at room temperature.
- If desired, a cooling pack can be placed underneath the source plate to keep source solutions cold.
- Humidity needs to be 50-70% during arraying to prevent evaporation from source plate.

2. Antibody Dilution

- Dilute capture antibodies in 1X PBS.
- Suggested capture antibody concentration is 0.1-1mg/ml.
- 20 μ l per source plate well is needed.
- Use a V-bottom 96-well plate, such as NUNC™ polystyrene 96-well V bottom plate (VWR catalogue No. 73520-486) as source plate.

3. Slide Arraying

- The mechanics and methodology of arraying using the Manual Glass Slide Microarrayer can be found in V&P Scientific, Inc. "Technical Note 42".
- When using nitrocellulose-coated slides, it is suggested that every other vertical indexing hole is used, rather than all 12. If all 12 vertical index holes are used, spots may spread into each other. All 8 horizontal indexing holes can be used.

Note: Although the pin diameter is 457 μ m, the spot size can be considerably larger because liquid spreads on nitrocellulose. Spot size depends on the nature of the source solution. Spot pitch is 750 μ m on the vertical axis and 1,125 μ m on the horizontal axis.

- After printing is complete, let slides air dry for 1 hour.

Assay And Detection

1. Blocking

- Block in Odyssey® Blocking Buffer for 1 hour at room temperature on a shaker with gentle agitation.

Note: Optimization of blocking buffer will be needed for best antibody performance.

- The incubation can be done in a plastic bag, hybridization chamber, or box.

2. Sample Binding

- Add protein sample diluted in Odyssey Blocking Buffer.
- Incubate on an orbital shaker at RT for 1-2 hours or overnight at 4°C.

3. Wash

- Wash 3 times with 1X PBST (0.1% Tween-20®), 5 minutes per wash.

4. Detection Using Biotinylated Detection Antibody

- Make a 1:500 dilution of biotinylated detection antibody in Odyssey Blocking Buffer.
- Incubate at RT for 1-2 hours on an orbital shaker.

Note: The detection antibody dilution must be optimized. A 1:500 dilution is a suggested starting point.

- Incubate the slides in diluted antibody for 1-2 hours at room temperature on an orbital shaker.
- Wash 3 times with 1X PBST (0.1% Tween-20), 5 minutes per wash.
- Make a 1:5000 dilution of 1mg/ml streptavidin-IRDye™ 800CW (LI-COR, Part # 926-32230) in Odyssey Blocking Buffer plus 0.1% Tween-20.
- Incubate the slides in diluted IRDye™ 800CW-streptavidin for 30 minutes at room temperature on a shaker in the dark.
- Wash 3 times with 1X PBST (0.1% Tween-20), 5 minutes per wash.
- After the last wash, scan the wet slide to see if the washing was sufficient (background is high with insufficient washing). If so, dry the slide by centrifugation. If not, wash one more time.

TIP: If a slide centrifuge is not available, the slide can be placed into a 50 ml conical tube and centrifuged at a low RPM for drying.

- Scan the slide on the Odyssey scanner with 42 µm resolution and an initial intensity setting of 5. If the signal is too strong or weak, rescan at a lower or higher intensity setting.

II. Tips For Lysate Arraying (Reverse Phase Array)

See V&P Scientific, Inc. "Technical Note 42" for a detailed description of the care and use of the Manual Glass Slide Microarrayer.

Cell Lysate Arraying

1. Getting Started

- Cell lysate arraying can be done at room temperature.
- If desired, place a cooling pack underneath the source plate to keep the source solutions cold.
- Humidity needs to be 50-70% during arraying to prevent evaporation from source plate.

2. Cell Lysate Preparation

- Use appropriate lysis buffer to prepare cell lysates.

Note: The lysis buffer MUST NOT contain any loading dye; loading dyes may be detected by the Odyssey.

- Boil lysates for 5 minutes, centrifuge at 14,000 rpm for 2 minutes, and store at 4°C.
- Immediately before arraying, transfer lysates to a source plate with two-fold serial dilutions to determine the best concentration for future arraying.
- 20 µl per source plate well is needed.

- Use a V-bottom 96 well plate, such as NUNC™ polystyrene 96 well V bottom plate (VWR catalogue No. 73520-486) as source plate.

3. Slide Arraying

- The mechanics and methodology of arraying using the Manual Glass Slide Microarrayer can be found in V&P Scientific, Inc. "Technical Note 42".
- It is suggested that every other vertical indexing hole is used rather than all 12. If all 12 vertical index holes are used, spots may spread into each other. All 8 of the horizontal indexing holes can be used.

Note: Although the pin diameter is 457 µm, the spot size can be considerably larger because liquid spreads on nitrocellulose. The spot size depends on the nature of the source solution. The spot pitch is 750 µm on the vertical axis and 1,125 µm on the horizontal axis.

- After printing is complete, let the slides air dry for 1 hour.

Assay And Detection

Note: It is highly recommended that the primary antibodies used for lysate array detection be validated by Western Blot analysis using the identical blocking and detection methods.

1. Blocking

- Block in Odyssey Blocker for 1 hour at room temperature on a shaker with gentle agitation.

NOTE: Optimization of blocking buffer will be needed for best antibody performance.

- The incubation can be done in a plastic bag, hybridization chamber, or box.

2. Primary Antibody Binding

- Make a 1:500 dilution of primary antibody in Odyssey Blocking Buffer plus 0.1% Tween-20.

Note: The Primary antibody dilution needs to be optimized. A 1:500 dilution is a suggested starting point.

- Incubate the slides in diluted antibody on an orbital shaker for 0.5-2 hours at room temperature or overnight at 4°C.

3. Wash

- Wash 3 times with 1x PBST (0.1% Tween-20), 5 minutes per wash.

4. Detection

- Make a 1:5000 dilution of 1mg/ml Secondary Antibody labeled with IRDye™ 800CW in Odyssey Blocking Buffer plus 0.1% Tween-20.

- Incubate slides in diluted antibody for 1 hour at room temperature on a shaker in the dark.

- Wash 3 times with 1x PBST (0.1% Tween-20), 5 minutes per wash.

- After the last wash, scan the wet slide to see if the washing was sufficient. If so, dry the slide by centrifugation. If not, wash one more time.

TIP: If a slide centrifuge is not available, the slide can be placed into a 50ml conical tube and centrifuged at a low RPM for drying.

- Scan the slide on the Odyssey scanner with 42 µm resolution and an initial intensity setting of 5. If the signal is too strong or weak, rescan at a lower or higher intensity setting.

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