Standard µArray Protocol

Glossary

1o – primary
2o – secondary
Ab – antibody
Ag – antigen
FCS – fetal calf serum (eliminates non-specific protein binding)
PBS – phosphate buffer saline (provides proper salinity)
Tween 20 – surfactant (eliminates non-specific non-polar binding)

ddH2O – double distilled water

Buffers

Blocking & Wash Buffer
3% FCS in 1X PBS

Sample & Antibody Dilution Buffer
3% FCS in 1X PBS

Figure 1 – Slide Orientation

Print Side

Label Side

1. Number Slides
   • Use diamond scribe to number slides on label side (see figure 1)
   • Make a hydrophobic outline around the spots on the print side with a hydrophobic pen (Pap Pen)

2. Block Slides
   • Incubate slides in blocking buffer overnight at 4°C (rotate at ~30 rpm – added after 10/30/03 print)

3. Put 1o Ab on slides
   • Place slides, print side up, into a tray for incubation
   • Put 300, 400, or 500 µl of diluted serum (test sample) on the print side directly on top of the array spots. If the hydrophobic outline was done properly, then fluid will stay inside the frame.
   • Incubate slides for 1 hour at 4°C (rotate at ~30 rpm – added after 10/30/03 print)

4. Wash #1
   • Perform a quick rinse in old wash buffer (blocking buffer used for overnight incubation)
   • Put slides into fresh wash buffer and place on shaker platform for 15 minutes at ~40 rpm
   • Repeat previous step

5. Put 2o Ab on slides
   • Place slides into a tray for incubation, print side up
   • Put 300, 400, or 500 µl of diluted 2o Ab (fluorescent marker) on the print side directly over the array spots. The fluorescent marker is photosensitive so cover with Al foil during the incubation to prevent photo bleaching.
   • Incubate slides for 45 minutes at 4°C (rotate at ~30 rpm – added after 10/30/03 print)

6. Wash #2
   • Perform a quick rinse in previous wash buffer
   • Put slides into fresh wash buffer and place on shaker platform for 30 minutes at ~40 rpm
   • Repeat previous step
   • Put slides into 1X PBS and place on shaker platform for 20 minutes at ~40 rpm
   • Repeat previous step
   • Put slides into ddH2O and shake for 15 seconds
   • Repeat previous step

7. Centrifuge to dry slides
   • Spin at 650 – 750 rpm for 8 minutes at 25°C (make sure centrifuge is balanced)
   • Put slides into slide box and store at 25°C (slides are ready to be scanned)