Coating antibodies (proteins) on Poly-Lysine glass slides

1. Place poly-lysine-coated glass slides in a 100-mm Petri dish with the coated surface face up. Label the top end of each slide with experiment name and number using a diamond pen.

CRITICAL STEP Slides should be handled with forceps to avoid scratching the coated surface.

2. Place 80 µl of capture antibody solution (12.5 µg/ml each Ab, upto 5 Abs at a time) in the center of each slide, and gently place a LifterSlip over the drop to spread the solution uniformly over a section of the slide. Alternatively, place the LifterSlip on the slide and deposit liquid at one end of the slip to fill the cavity by capillarity.

CRITICAL STEP Mix the solution of capture antibodies thoroughly before use, and be sure to note the area of the slide over which the secondary antibodies are deposited; microarrays will only form within this region.

3. Incubate slides in a humidified container (e.g., a Petri dish or a plastic box with a dampened Kimwipe or with water sprayed on the lid) at room temperature for 1 h.

PAUSE POINT Alternatively, incubation can be carried out overnight at 4 C in a humidified container.

4. Add 50 ml of blocking buffer (3% milk/PBST) to the Petri dish containing the slides. The slips will float off the slides. Carefully remove the slips without contacting the surface of the slides. It may be necessary to gently nudge the LifterSlip at one edge or corner, with a pair of forceps, to release the slip from the slide.

5. Place the Petri dish on a bench-top shaker and block slides for 10 min at room temperature.

6. Decant blocking buffer and replace with 30 ml of PBST to remove unbound proteins. Wash slides 10 min.

7. Decant PBST solution and replace with PBS (30 ml). Wash slides for 5 min.

8. Dip each slide once in a 30-ml of deionized water. DO NOT leave slide in water

9. Dry slides in a microarray centrifuge and keep in a humidified container (e.g., slide box, Petri dish) until microengraving.

CAUTION Do not open centrifuge until moving parts come to a stop.

PAUSE POINT Slides processed to this stage can be stored for use the following day in a humidified chamber kept at 4 C.