

Example Protocol for Protein Array s

1. Preparation of Targets for Printing

- A. Dissolve proteins in 1xPBS with 0.05% BSA (pH: ~7.4), at room temperature to a final concentration of ~100 μ g/mL.
- B. Transfer the targets to a 96 or 384 spotting plate with volume of ~20 μ L.
- C. Gently shake the plate to bring liquid to the bottom of wells.
- D. Now you can set up array spotter and spot slides.

2. Humidity

- A. After spotting, place the printed slides in a chamber with relative humidity of 65-75% for 10 to 16 hours. To make a humidity chamber with 65-75% humidity, place saturated sodium chloride solution (such as 100g of NaCl solids in 40~50mL Milli-Q H₂O) in a regular chamber with tight seals.
- B. Upon removing the slides from the humidity chamber, allow the slides to dry at room temperature for 30 minutes.

3. Pre-Treatment

- A. Prepare the pre-treatment solution: 1xPBS/1% BSA.
- B. Incubate the slides in the pre-treatment solution for 30 minutes at room temperature on an orbital shaker.
- C. Remove the slides and rinse them extensively with Milli-Q water and blow dry with a gentle stream of nitrogen.

4. Binding of Labeled Samples

- A. Clean glass coverslips with 70% ethanol, and dry with nitrogen.
- B. We recommend using 30 μ L of sample mixture when a full coverslip (24 mm x 60 mm) is used.
- C. Determine the appropriate concentration for labeled samples. The concentration may vary depending upon the makeup of the experiment and the complexity and affinity of the labeled samples.
- D. Dissolve labeled protein samples in the diluent of your choice.
- E. Place 30 μ L of the labeled sample mixture on each slide and lay down the coverslip (Avoid any bubbles under the coverslip).
- F. Carefully place the slides into a humidified chamber with 100% humidity.
- G. Incubate at room temperature for about 2 to 4 hours.

5. Washes

- A. Wash the slides 3 times with TBS/0.1% Tween at room temperature. (5 minutes per wash.)
- B. Wash the slides 2 times with TBS at room temperature. (5 minutes per wash.)
- C. Dip the slides thoroughly in a staining jar containing Milli-Q water at room temperature and then dry slides with a gentle stream of nitrogen immediately.
- D. Slides are ready for scanning.