

## **Example Protocol for Protein Arrays**

### **1. Preparation of Printing Contents**

- A. Dissolve the printing contents in FMB Protein Printing Buffer (P/N: ABP01), 1x PBS (pH=7.4), or 1x PBS/0.05% BSA (pH=7–8), at room temperature, to a desired concentration:
  - Antibodies: 0.5 mg/mL or higher
  - Proteins: 100 µg/mL or higher
  - Peptides: 0.1–0.5 mg/mL
  - Cell lysate: to be determined based on your need
- B. Transfer the targets to a 96 or 384 spotting plate with volume of ~20 µL.
- C. Gently tap the plate to bring liquid to the bottom of wells.
- D. Follow the array spotter manufacturer's instructions to set up the array spotter, load slides and plates.
- E. Print the arrays.

### **2. Humidity Treatment**

- A. After spotting, incubate the printed slides in a chamber with relative humidity of 65-75% for 8 to 16 hours.

Note: A humidity chamber with 65-75% humidity may be constructed in your laboratory. To do so, find a two-shelf chamber with tight seals. Make sure the chamber is large enough to accommodate the number of slides that will be placed inside. In a shallow open container, add 100 g of NaCl solids to 40~50 mL Milli-Q H<sub>2</sub>O. The amount of salt solution needed may vary depending on the size of the chamber. Place the container with the salt at the bottom of the chamber. Close the chamber door.
- B. Upon removing the slides from the humidity chamber, allow the slides to dry in a low humidity environment (35% or less) at room temperature for 30 minutes to an hour.

### **3. Pre-Treatment**

- A. Prepare pre-treatment solution: 1x PBS/2% nonfat milk or 1x PBS/0.05–0.1% BSA.
- B. Incubate the slides in pre-treatment solution for 30 minutes at room temperature or 4°C 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. Coupling with Samples or Serum**

- A. Clean glass coverslips with 70% ethanol, and dry with nitrogen.
- B. We recommend using 30 to 50 µL of sample mixture when a full coverslip (24 mm x 60 mm) is used.



- C. Determine the appropriate concentration for your samples. The concentration may vary. When necessary, dilute your samples with 1x PBS/3–5% BSA, 1x PBS/1–2% nonfat milk, or 1x PBS.
- D. Place 30 to 50  $\mu$ L of the diluted sample mixture on each slide and lay down the coverslip (Avoid any bubbles under the coverslip).
- E. Carefully place the slides into a humidified chamber with 100% humidity.
- F. Incubate at 4°C, room temperature or 37°C depending on your needs, for 1 to 2 hours or more if desired.
- G. Washes
  - 1. Wash the slides three times with TBS/0.05–0.1% Tween, or 1x PBS/0.05–0.1% Tween at room temperature. (10 to 15 minutes per wash)
  - 2. Dip the slides two times in 0.2x SSC in a staining jar at room temperature.
  - 3. Rinse the slides thoroughly with Milli-Q water at room temperature and then dry slides with a gentle stream of nitrogen immediately.

## **5. Detection with Labeled Samples**

- A. Clean glass coverslips with 70% ethanol, and dry with nitrogen.
- B. We recommend using 30 to 50  $\mu$ 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 1x PBS/3–5% BSA, 1x PBS/1–2 % nonfat milk, or 1x PBS.
- E. Place 30 to 50  $\mu$ 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 or 37°C or 4°C for 2 to 4 hours.
- H. Washes
  - 1. Wash the slides three times with TBS/0.05–0.1% Tween, or 1x PBS/0.05–0.1% Tween at room temperature. (10 to 15 minutes per wash.)
  - 2. Dip the slides two times in 0.2x SSC at room temperature.
  - 3. Rinse the slides thoroughly with Milli-Q water at room temperature and then dry slides with a gentle stream of nitrogen immediately.
- I. Slides are ready for scanning.