Hybridization Protocol

Product Name: ***Matrix II Slides***
Catalog No.: CPX 5, CPX 25, CPX 50

This protocol has been optimized to allow users to produce arrays of cDNA or oligonucleotides dissolved in 50% DMSO, 3x SSC, or 150 mM sodium phosphate buffer.

1. **Preparing targets for spotting**
   A. We recommend dissolving cDNA, unmodified oligos or amino-modified oligos in 50% DMSO/50% H_2O, 3x SSC, or 150 mM sodium phosphate buffer.
   B. Transfer the targets to a 96 or 384 spotting plate with a volume of ~20 μL/well.
   C. Gently shake the plate to bring liquid to the bottom of the wells.
   D. Now you can set up the array spotter and start printing slides.

2. **UV Cross-linking**
   A. After spotting, allow the slides to dry for 30 minutes at room temperature.
   B. UV cross-link the slides at **400mJ when working with cDNA**, or **600mJ when working with oligos**. **Important**: Do not omit the step!

3. **Pre-Treatment**
   A. Prepare and preheat Pre-treatment Solution (2x SSC/0.2% SDS/0.1% BSA) to 55 ^{o}C.
   B. Place the slides in a slide rack.
   C. Place the slides in a staining jar with Pre-treatment Solution for 20 to 30 minutes at room temperature on an orbital shaker.
   D. Remove the slides from the pre-treatment solution and **rinse thoroughly** with Milli-Q water.
   D. Dry the slides with a gentle stream of nitrogen.
   E. Then allow the slides to dry at room temperature for 15 – 30 minutes.

4. **Preparation of probes** (Be sure to suspend the probes in FMB Hybridization Buffers)
   A. We recommend using 35 μL of hybridization mixture for each full slide.
   B. Spin-dry cDNA or oligo probes in a Speedvac.
   C. For hybridization of each slide, resuspend the probes in 3 μL of nuclease free water.
   D. Quickly vortex the probes and centrifuge it for 30 seconds.
   E. Denature the probes on a heat-block at 90 – 95°C for 3 – 5 minutes.
   F. Remove the probe mix from the heat-block and immediately place it on ice.
   G. Add 32 μL of hybridization buffer to probes. Please use FMB cDNA Hybridization Buffer (P/N: HBC 01) when working with cDNA or FMB Oligo Hybridization Buffer (P/N: HBO 01) when working with oligos.
   H. Place the mixture on ice for five minutes before applying it to slides.

5. **Hybridization**
   A. We recommend using 35 μL of the probe mix for each slide when using a full coverslip (24 mm X 60 mm).
   B. Clean coverslips with 70% ethanol, and blow-dry with nitrogen.
   C. Quickly vortex the probe mixture before applying it to the printed slides.
   D. Place 35 μL of the probe mixture on each slide and carefully lay down the coverslip (Be careful – avoid air bubbles under the coverslip).
   E. Incubate the slides in a humidified chamber with 100% humidity, at 42 ^{o}C or the temperature suitable for your samples for 12 – 14 hours.

6. **Washing the slides after hybridization**
   A. Preheat Wash Solution 1 (0.2x SSC, 0.2% SDS) and Wash Solution 2 (0.2x SSC) to 55 ^{o}C.
   B. Remove cover slips by quickly rinsing the slides with running DI water, and then place the slides in a slide rack.
   C. Immerse the slides in Wash Solution 1 for 20 minutes at room temperature on an orbital shaker.
   D. Transfer the slides to a staining dish with Wash Solution 2 and gently dip the slides up and down for one minute.
   E. Repeat twice. Be sure to use **fresh** Wash Solution 2 each time.
   F. **Thoroughly rinse the slides three times with fresh Milli-Q water** at room temperature.
   G. Dry slides with a gentle stream of nitrogen immediately.
   H. The slides are now ready for scanning.