



**FULL MOON**  
*BioSystems*

# FMB *cDNA* Slides

- User Guide -

**Product description**

FMB cDNA Slides are designed to immobilize cDNA for microarray applications. The slides are coated with Full Moon BioSystems' proprietary materials. The slide surface is optimized to increase immobilization efficiency, improve spot uniformity and morphology, and enhance array reproducibility.

Either side of a slide may be used. If the slides come with bar-code labels, please use the side with bar-code labels.

**Storage and handling**

Handle with care. Use the slides in a clean environment. Avoid direct contact with the slide surface. Any foreign material may affect the quality of arrays to be printed on the slides.

Store desiccated at room temperature.

**Safety and warning**

For research only. Not recommended for clinical diagnostic uses unless otherwise instructed. Do not use on human or animals.

**Support**

For support, please call 1.877.316.7308 or 408.737.1702, or email your questions to [support@fullmoonbiosystems.com](mailto:support@fullmoonbiosystems.com).

**Materials supplied by users**

- cDNA Printing Buffer (Full Moon BioSystems, Inc., P/N: CPS 01, 25mL)
- 50% DMSO/50% H<sub>2</sub>O
- UV cross-linker
- Slide racks
- Staining jars or containers
- Deionized water
- Nuclease free water
- Compressed nitrogen or centrifuge
- Speed Vac
- Orbital shaker
- Heat block at 90 – 95 °C
- Ice
- Coverslips
- Incubator with 100% relative humidity at 42 °C, commercially available or homemade. (A homemade incubator can be put together in your lab. Add 50 mL water in shallow plastic tray. Place the tray at the bottom of a two-shelf container with tight seals. The amount of water may vary dependent upon the size of the container. Place the unit into an oven or incubator set at 42 °C. This forms an environment with 100% relative humidity.)
- Post-printing/Pre-hybridization treatment solution
  - 2x SSC/0.1% SDS/1% BSA, preheated to 55 °C
- FMB cDNA Hybridization Buffer (Full Moon BioSystems, Inc., P/N: FMB HBC 01, 500µL)
- Post-hybridization processing
  - Wash Solution 1: 0.2x SSC/0.2%SDS, pre-heated to 55 °C
  - Wash Solution 2: 0.2x SSC, pre-heated to 55 °C

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## **Protocol**

### **1. Preparing targets for printing**

- a. Dissolve targets to a concentration  $\sim 0.25 \mu\text{g}/\mu\text{L}$  in FMB cDNA Printing Buffer (P/N: CSP 01), or 50% DMSO/50% H<sub>2</sub>O (in volume) at room temperature.
- b. Transfer the targets to a 96 or 384 spotting plate.
- c. Gently tap the plate to bring liquids to the bottom of wells.
- d. Set up array spotter and print slides according to manufacturer's protocol.

### **2. UV Cross-linking**

- a. After spotting, UV cross link the slides at 400 mJ.  
**Note:** This step is required to ensure proper immobilization of spotted samples. Please do not omit!
- b. Allow the slides to dry at room temperature for 30 minutes.  
**Note:** If you plan to store the printed arrays for future use, stop here and follow the instructions to prepare the slides for storage.  
**Storage of printed slides:** Place the printed slides in a clean slide box. Insert the slide box into a plastic bag and seal the bag securely to prevent moisture. The slides may be store at 2 – 8 °C for 3 to 6 months.

### **3. Pre-hybridization treatment**

- a. Prepare pre-treatment solution (2x SSC/0.1% SDS/1% BSA) and pre-warm it to 55 °C.
- b. Transfer the slides to a slide rack.
- c. Place the slides in a staining jar on an orbital shaker. Immerse the slides with the pre-treatment solution for 20 to 30 minutes at room temperature.
- d. Remove the slides from the staining jar. Rinse thoroughly with deionized water.
- e. Dry the slides by centrifugation or with a gentle stream of compressed nitrogen (approx. 20-30 psi.)

#### 4. Preparation of probes

**Note:** We recommend using 35  $\mu$ L of hybridization mixture for each hybridization reaction on a slide with a full-size coverslip (60mm x 24mm). The amount may vary depending on the size of the printed area and coverslip.

- a. Determine the amount of labeled probes to be used for each reaction. Typically 2 – 5  $\mu$ g of probes are used for each hybridization reaction when a full-size coverslip is used.
- b. Spin dry the probes in a Speed Vac.
- c. For each reaction, re-suspend probes in 3  $\mu$ L of nuclease free water.
- d. Quickly vortex and centrifuge for 30 seconds.
- e. Denature the probes on a heat-block at 95 °C for 5 minutes.
- f. Remove the probe mixture from the heat-block and immediately place it on ice.
- g. Add 32  $\mu$ L of FMB cDNA Hybridization Buffer (P/N: FMB HBC 01) to the probes.
- h. Preserve the mixture on ice.

#### 5. Hybridization

- a. Quickly vortex the probe mixture before applying it to the printed slides.
- b. Place 35  $\mu$ L of the probe mixture on each slide.
- c. Carefully place a coverslip on top of the arrays. Use extra care – avoid air bubble formation under the coverslip.
- d. Incubate the slides in a humidified chamber with 100% humidity for 12 to 18 hours at 42 °C or the temperature suitable for your samples.

#### 6. Post-hybridization processing

**Note:** Do not allow the slides to dry between washes.

- a. Prepare and preheat Wash Solution 1 (0.2x SSC, 0.2% SDS), and Wash Solution 2 (0.2x SSC) to 55 °C.
- b. Remove cover slips by quickly rinsing the slides with running deionized water. Transfer the slides to a slide rack.
- c. Place the slides in a staining jar or container set on a orbital shaker. Immerse the slides in Wash Solution 1. Shake for 20 minutes at room temperature.

- d. Transfer the slides to a second staining dish with Wash Solution 2. Gently dip the slides up and down for one minute at room temperature. Repeat twice. Be sure to use **fresh** wash solution each time.
- e. Rinse the slides three to five times with fresh deionized water at room temperature.  
**Note:** It is crucial to remove all SDS from slide surface. Any SDS residue may cause high background fluorescence. Please be sure to wash the slides thoroughly.
- f. Dry the slides immediately with a gentle stream of compressed nitrogen (approx. 20 – 30 psi) or by centrifugation.
- g. The slides are now ready for scanning.