

FMB cDNA Hybridization Buffer

Catalog number: HBC01, 02, 03

Overview

FMB cDNA Hybridization Buffer is used to dissolve labeled probes for hybridization. The buffer contains unique elements that promote sample diffusion on the slide, increasing hybridization efficiency and consistency. It also works to reduce background fluorescence.

Component: cDNA Hybridization Buffer

Storage condition: -4°C

Protocol

1) Prepare hybridization solution and probes

- a) We recommend using 30 pmole of probes and 35 μ L of hybridization mixture for each full slide.
- b) Spin-dry the cDNA probes in a Speedvac.
- c) For hybridization of each slide, resuspend the probes in 3 μ L 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 from the heat-block and immediately place them on ice.
- g) Add 32 μ L of *cDNA Hybridization Buffer* (Full Moon Biosystems, Inc., P/N: HBC 01) to the probes.
- h) Place the mixture on ice for five minutes before applying it to slides.

2) Hybridization

- a) We recommend using 35 μ L 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 μ L of the probe mixture on each slide and carefully place a coverslip on top of the arrays. (Use extra care – avoid air bubbles.)
- e) Incubate the slides in a humidified chamber with 100% humidity at 42 °C for 12 to 18 hours.

3) 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 °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. Repeat twice. Be sure to use **fresh** Wash Solution 2 each time.
- e) **Thoroughly** rinse the slides three times with fresh DI water at room temperature.
- f) Dry the slides immediately with a gentle stream of compressed nitrogen or by centrifugation.
- g) The slides are now ready for scanning.