

FMB Oligo Hybridization Buffer

Catalog number: HBO01, 02, 03

Overview

FMB Oligo 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: Oligo Hybridization Buffer

Storage condition: -4°C

Protocol

1. Preparation of probes

- a) We recommend using 35 μ L of hybridization mixture for each hybridization reaction on a slide with a full-size coverslip (60mm x 24mm). The amount of mixture may vary depending on depending on the size of the printed area and coverslip.
- b) Spin dry the probes in a Speed Vac.
- c) For hybridization on each slide, resuspend the probes in 3 μ L of nuclease free water.
- d) Quickly vortex the probes and centrifuge for 30 seconds.
- e) Denature the probes on a heat-block at 95°C for 5 minutes.
- f) Remove the probe mix from the heat-block and immediately place it on ice.
- g) Add 32 μ L of FMB Oligo Hybridization Buffer (P/N: HBO 01) to the probes.
- h) Preserve the mixture on ice before applying it to slides.

2. Hybridization

- a) Quickly vortex the probe mixture before applying it to the printed slides.
- b) Place 35 μ L of the probe mixture on each slide.
- c) Carefully place a clean coverslip on top of the arrays. Use extra care – avoid air bubble formation under the coverslip.
- d) Incubate the slides in a chamber with 100% humidity at 42 °C or at the temperature suitable for your samples for 12 – 14 hours.

3. Washing the slides after hybridization

Note: Do not allow slides to dry between washes.

- a) Preheat Wash Solution 1 (0.2x SSC, 0.2% SDS), Wash Solution 2 (0.2x SSC) to 55 °C.
- b) Remove cover slips by quickly rinsing the slides with running DI water. Place the slides in a slide rack.
- c) Place the slides in a staining dish or container set on an orbital shaker. Immerse the slides with Wash Solution 1. Shake for 20 minutes at room temperature.
- d) Transfer the slides to a second 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 each time.
- e) Thoroughly rinse the slides 3 to 5 times with **fresh** deionized water at room temperature.
 - i) **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 psi) or by centrifugation.
- g) The slides are now ready for scanning.