PRODUCT: **METHYLENE BLUE STAIN** June 2014
Cat. No: MB 119
Storage: Store at room temperature. The product is stable for at least one year after the date of purchase.

PRODUCT DESCRIPTION

METHYLENE BLUE STAIN is an aqueous solution of methylene blue designed for quantitative or qualitative examination of RNA and DNA immobilized on hybridization membranes in northern, Southern and alkaline blotting. Unlike ethidium bromide (a potent carcinogen), METHYLENE BLUE STAIN does not interfere with retention of RNA and DNA on the hybridization membrane nor with the hybridization process (1). METHYLENE BLUE STAIN was tested and found applicable for use with both plastic and nitrocellulose membranes. However, nitrocellulose membranes exhibit higher background staining.

1. Herrin, D. L. and Schmidt, G. W. (1988). Rapid, Reversible Staining of Northern Blots Prior to Hybridization. Biotechniques, 6, 196-200.

STAINING PROTOCOL

A. Staining before hybridization.

1. Immobilize RNA or DNA on a hybridization membrane according to the manufacturer's directions.
2. Immerse the membrane (dry or wet) in METHYLENE BLUE STAIN and carry out staining for 5 - 10 minutes at room temperature.
3. Pour METHYLENE BLUE STAIN back into a bottle (the stain is reusable) and wash the membrane three times with water, each time gently shaking the membrane for 5 - 10 seconds.
4. RNA and DNA bands stain blue against the white to bluish background of the membrane. METHYLENE BLUE STAIN detects > 20 ng RNA or DNA/band.
5. The stain can be completely removed from the membrane prior to hybridization by washing with ethanol (75 - 100 %) or SDS (0.1 - 1.0 %). After washing with ethanol or SDS, wash the membrane with water for 2 - 3 minutes.

B. Hybridization

In a typical hybridization protocol, METHYLENE BLUE STAIN is removed from the membrane with a pre-hybridization solution containing SDS. For staining of membranes exposed to SDS, wash the membranes with ethanol, as described below.

C. Staining after hybridization.

Membranes that have been exposed to SDS during hybridization should be washed with ethanol prior to additional staining. To re-stain membranes exposed to SDS, wash the membranes with ethanol as described here.

1. Immerse the membrane in a tray with ethanol (75 - 100 %) and carry on washing for 5 - 15 minutes with occasional shaking.
2. Pour off ethanol and wash the membrane for 2 - 3 minutes in water.
3. Stain the washed membrane as described in the above staining protocol.

QUANTITATION OF RNA OR DNA.

Wet the membrane and a piece of blotting paper with water. Place the membrane on wet paper, photograph and use either a negative or positive image for a densitometer scanning. For fast results, use Polaroid film type 667, 665 or 55.

