

TMB Blotting

CHROMOGENIC SUBSTRATES

Precipitating HRP Substrate

Cat. no. 4450

Product Characteristics

TMB (3, 3', 5, 5'-tetramethylbenzidine) is a chromogenic substrate for Horseradish Peroxidase (HRP). TMB produces a deep blue color during the enzymatic degradation of hydrogen peroxide by HRP.

TMB Blotting liquid ready-to-use substrate is a highly active and stable blotting substrate utilized for measuring HRP probe activity. A stable blue precipitate is formed at the reaction site.

The substrate does not contain NMP (1-methyl-2-pyrrolidone) making it REACH Restricted Substances List Annex XVII compliant, while ensuring maximal safety during use, and minimal waste problems after use.

Composition & Properties

Ready-to-use substrate: Includes substrate buffer and hydrogen peroxide. No other reagents should be added.

Working Procedure

The following procedure is applicable to nitrocellulose membranes. The procedure must be optimized for other membranes.

1. The desired amount of substrate is poured into a sealed container and allowed to reach room temperature in the dark before use.
2. In order to prevent the blotted membrane from fading, we recommend the use of 0.1M K₂HPO₄, pH 7.2 in coating.

3. After the last incubation with HRP-labelled Streptavidin or HRP-labelled secondary antibody, it is recommended to wash the membrane in a 0.1 M Tris buffer pH 7.4.
4. Shake off the excess buffer and incubate the membrane in the TMB Blotting solution for 10 minutes.
5. Wash the membrane in distilled water and allow it to dry.
6. The site of positive reaction will appear light blue with no or very little background staining.

Tips & Tricks

- The membrane can be blocked, prior to applying the substrate, with Kementec's Synthetic Blocking Buffer for Blotting (cat. no. 4650).
- For long-term preservation of the results, the membranes must be stored in the dark.

Handling & Storage

- Store solution at 2-8 °C in the dark.
- Avoid exposure to light, heat and contamination with metal ions or peroxidase
- Re-dispense only into bottles made of High-Density Polyethylene (HDPE), amber color. Dispensing guidelines are available upon request.



Our Immunoassay Solutions are eco-friendly, creating a healthy work environment and preserving natural resources, while helping our customers fulfill significant regulatory requirements. **We call it ECO-TEK.**