

# 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 KH<sub>2</sub>PO<sub>4</sub>, 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 or block the membrane if desired 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 washed in distilled water.
- 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 environmentally conscious, creating a safer work environment while helping our customers fulfill significant regulatory requirements.

**We call it ECO-TEK!**



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