

TMB ONE/TMB ONE Prestained

CHROMOGENIC SUBSTRATES

ELISA HRP Substrate

Cat. No. 4380/4430

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, and the addition of a stop solution gives a clear yellow color that absorbs at 450 nm.

TMB ONE is a ready-to-use, very active and stable aqueous formulation. It 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

1. The desired amount of substrate is poured into a sealed container and allowed to reach room temperature in the dark before use.
2. A recommended volume of 100 µl substrate solution per microtiter well is added. Time of development is typically 5-30 minutes. We recommend the plate to be kept in the dark during incubation.
3. In kinetic assays, the blue color is read directly at 650 nm. For endpoint assays 100 µl (equal volumes) of 0.20 M sulphuric acid is added and the absorbance is read at 450/620 nm. The yellow solution is stable for at least one hour.

Tips & Tricks

- If reduced intensity is required, it is recommended to shorten the time of development or to dilute the conjugate/antibody in the immunoassay further.
- Dilution of the substrate is not recommended unless a corresponding TMB ONE Diluent is acquired.
- For traceable pipetting, we recommend using the pink colored TMB ONE Prestained (cat. no. 4430). The pink indicator disappears after addition of the stop solution.
- In case of incidents of precipitation with very high OD-values, we recommend the use of the TMB X-tnd (cat. no. 5280)

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.**