

TMB PLUS2[®]/TMB PLUS2[®] Prestained

CHROMOGENIC SUBSTRATES

ELISA HRP Substrate

Cat. no. 4395/4445

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 PLUS2 is a ready-to-use, very active and stable aqueous formulation and this substrate gives higher signal than standard TMB solutions ensuring a very fast assay reaction. 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 negative environmental impact.

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. Keep the plate 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 PLUS2 Diluent is acquired. The diluted TMB solution maintains the same stability performance as the undiluted version, and a specific activity can be achieved.
- For traceable pipetting, we recommend using the pink colored TMB PLUS2 Prestained (cat. no. 4445). 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.**