

Product Information

3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Supersensitive, for ELISA ready to use solution

Catalog Number **T4444**
Storage Temperature 2–8 °C

Product Description

3,3',5,5'-Tetramethylbenzidine (TMB) is a chromogenic substrate suitable for use in ELISA procedures, which utilize horseradish peroxidase (HRP) conjugates.¹⁻⁴ This substrate produces a soluble end product that is blue in color and can be read spectrophotometrically at 370 or 650 nm. The reaction may be stopped with acid, resulting in a yellow solution that is read at 450 nm.

This product is supplied as a ready to use, one-component HRP substrate containing TMB in a mildly acidic buffer. Rate kinetics are ~40% faster than traditional TMB formulations. Prior to the reaction with HRP, the substrate should be a colorless to light bluish-green solution. The substrate system develops a blue reaction product when reacted with HRP in microwell applications. For end-point assays, acid can be used to stop the reaction, to yield a yellow end product. Since this substrate produces a soluble reaction product, it is not recommended for histochemistry or blotting.

Several publications cite use of this product in their protocols.⁵⁻⁸

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2–8 °C. This substrate is light sensitive and should be protected from direct sunlight or UV sources.

Procedure

1. Bring to room temperature before use.
2. Following the reaction with HRP, a blue reaction product forms that may be read at 370 nm or between 620 and 655 nm.
3. For end-point assays, the reaction can be stopped by the addition of a volume of 1 or 2 M HCl, or 0.5 M H₂SO₄, equal to the volume of the substrate reaction in the well. The resulting yellow end product, which is stable for at least one hour, can then be read at 450 nm. A preformulated Stop Reagent, Catalog Number S5814, is available for this application at 450 nm.
4. End-point assays can also be read at 650 nm using another Stop Reagent, Catalog Number S5689.
5. To reduce the intensity of a reaction, it is suggested that the antibodies or conjugates be diluted.

References

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AGW,GCY,MAM 02/19-1