Product Information

3,3',5,5'-Tetramethylbenzidine Liquid Substrate
Slow Kinetic Form, for ELISA
Peroxidase substrate

Catalog Number T4319
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. This substrate produces a soluble end product that is blue in color and can be read spectrophotometrically at 370 or 655 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 peroxidase substrate containing TMB in a mildly acidic buffer. Rate kinetics are ~25% slower than traditional TMB formulations. Prior to the reaction with HRP, the substrate should be a colorless to light bluish-green solution. Since this substrate produces a soluble reaction product, it is not recommended for histochemistry or blotting.

Several references cite use of this product in their protocols.

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 M or 2 M HCl, or 1 M H₂SO₄, equal to the reaction volume in the well. The resulting yellow end product, which is stable for at least one hour, can then be read at 450 nm. Sigma-Aldrich offers a preformulated stop solution (Catalog Number S5814) for this application. End-point assays can also be read at 650 nm using another Sigma-Aldrich stop solution (Catalog Number S5689).
4. Dilution of the substrate is not recommended. To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted.

References

AGW,GCY,MAM 02/19-1