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Product Information

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

Peroxidase substrate

T4319

Product Description

$$H_3C$$
 CH_3 H_2N NH_2 CH_3

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 TMB-HRP reaction produces a soluble end-product that is blue in color and can be read spectrophotometrically at 370 nm or 655 nm. The reaction may be stopped with acid, resulting in a yellow solution that is read at 450 nm.

This product is a ready-to-use, one component HRP substrate which contains 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 publications.^{5,6} theses⁷ and dissertations⁸⁻¹⁰ have cited use of product T4319 in their research.

Precautions and Disclaimer

This product is 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 this product at 2-8 °C. This product 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 N (0.5 M) H_2SO_4 , 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. A preformulated Stop Reagent (Cat. No. S5814) is available for this application. End-point assays can also be read at 650 nm using another Stop Reagent (Cat. No. S5689).
- 4. Dilution of the substrate is not recommended. To reduce the intensity of a reaction, dilution of the antibodies or conjugates is recommended.

References

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