

Product Information

3,3',5,5'-Tetramethylbenzidine Dihydrochloride

tablet, 1 mg substrate per tablet

Catalog Number **T3405**

Storage Temperature 2–8 °C

Product Description

3,3',5,5'-Tetramethylbenzidine (TMB) dihydrochloride is a peroxidase substrate suitable for use in ELISA procedures.¹⁻⁵ 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 2 M H₂SO₄, resulting in a yellow solution that is read at 450 nm.

Each tablet contains 1 mg of TMB dihydrochloride substrate. The product is available in packages of 5, 50, or 100 tablets per box, individually foil wrapped for ease of use, storage, and safety. Custom packaging and bulk purchase information are available upon request.

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

0.05 M Phosphate-Citrate Buffer

- Dissolve one phosphate-citrate buffer tablet (e.g., Catalog Number P4809) in 100 mL of ultrapure water with stirring, to yield a 0.05 M phosphate-citrate buffer, pH 5.0.
- Or**
- Add 25.7 mL of 0.2 M dibasic sodium phosphate (e.g., Catalog Number S0876), 24.3 mL of 0.1 M citric acid (e.g., Catalog Number C7129) and 50 mL of ultrapure water. Adjust the pH to 5.0, if necessary.

TMB Substrate Solution

- Dissolve one TMB dihydrochloride tablet in 1 mL of DMSO, and add to 9 mL of 0.05 M Phosphate-Citrate Buffer, pH 5.0. Add 2 µL of fresh 30% hydrogen peroxide (e.g., Catalog Number H1009) per 10 mL of substrate buffer solution, immediately prior to use.

Or

- Dissolve one TMB dihydrochloride tablet in 1 mL of DMSO, and add to 9 mL of 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.03% sodium perborate (capsules, Catalog Number P4922).

Stop Solution

The reaction may be stopped by the addition of 50 µL of 2 M H₂SO₄ per 200 µL of reaction mixture.

Storage/Stability

Store the TMB dihydrochloride tablets at 2–8 °C. Protect from heat, light, and moisture. Allow tablets to reach room temperature prior to use.

References

1. Liem, H.H. *et al.*, *Anal. Biochem.*, **98(2)**, 388-393 (1979).
2. Rosenqvist, E. *et al.*, "Determination of Antibody Responses to Meningococcal Antigens by ELISA", in *Meningococcal Vaccines: Methods and Protocols* (A.J. Pollard and M.C.J. Maiden, eds.). *Methods in Molecular Medicine*, Springer/Humana Press, pp. 255-273 (2001).
3. Fulton, S.A. *et al.*, *J. Infect. Dis.*, **178(4)**, 1105-1114 (1998).
4. Slocombe, L.L., and Colditz, I.G., *Food Agr. Immunol.*, **22(2)**, 135-143 (2011).
5. Ucisik, M.H. *et al.*, *Colloids Surf. B Biointerfaces*, **128**, 132-139 (2015).

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