

Product Information

3,3',5,5'-Tetramethylbenzidine

≥98% (TLC)

T2885

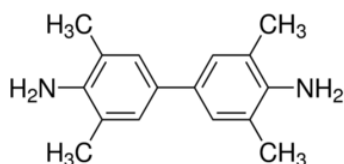
Product Description

CAS Registry Number: 54827-17-7

Molecular Formula: C₁₆H₂₀N₂

Formula Weight: 240.34

Structure:



3,3',5,5'-Tetramethylbenzidine (TMB) is a colorimetric substrate that is used with peroxidase (HRP) and peroxidase-coupled systems.¹ The reaction of TMB with peroxidase produces a soluble end product,² which is blue in color and can be read spectrophotometrically at 370 nm or 655 nm. The reaction can be stopped with 2 M sulfuric acid and read at 450 nm. This results in a 2-fold to 4-fold enhancement of the sensitivity of the reaction.

This T2885 product is the free base form of TMB. The hydrochloride salt of TMB can be directly dissolved in aqueous media. For this reason, the hydrochloride salt is the frequently used form of TMB. However, both the free base and the hydrochloride salt forms of TMB will give identical results.

TMB has been used in the quantitative determination of hemoglobin and cytochemical staining for peroxidase.³ Several publications,⁴⁻⁵ theses⁶⁻⁷ and dissertations⁸⁻¹³ have cited use of T2885 in their research 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 the product at 2-8 °C. It remains active for a minimum of two years as supplied.

Preparation Instructions

A TMB stock solution can be prepared by dissolving 1 mg/mL in DMSO. The working solution is made by diluting 1 mL of the DMSO stock with 9 mL phosphate-citrate buffer, pH 5.0, and then adding 2 µL of fresh 30% hydrogen peroxide (Cat. No. H1009) per 10 mL of substrate/buffer solution immediately prior to use.

Stock solutions of TMB in DMSO can be frozen in 1-2 mL aliquots at -20 °C. Solutions appear to remain active for at least two years when stored in this manner. Any oxidation would be revealed as a color change in the working solution.

References

- Holland, V.R. *et al.*, *Tetrahedron*, **30(18)**, 3299-3302 (1974).
- Josephy, P.D. *et al.*, *J. Biol. Chem.*, **257(7)**, 3669-3675 (1982).
- Liem, H.H. *et al.*, *Anal. Biochem.*, **98(2)**, 388-393 (1979).
- van Diemen, P.M. *et al.*, *Vet. Immunol. Immunopathol.*, **41(3-4)**, 307-321 (1994).
- Moon, H.K. *et al.*, *Asian-Aus. J. Anim. Sci.*, **12(2)**, 180-185 (1999).
- Patterson, E.K. *et al.*, *Free Radic. Biol. Med.*, **70**, 167-173 (2014).
- Medved, Sara, "Novi markeri za praćenje cijeljena kosti u bolesnika s prijelomom duge kosti" ("New markers for monitoring healed bone in patients with long bone fractures"). University of Zagreb, M.Sc. thesis, p. 7 (2014).

8. Gonçalves, Márcia Figueiredo, "Understanding how dendritic cell glycans affect antitumor immune responses". Universidade Nova de Lisboa, M.Sc. thesis, p. 72 (2015).
9. Kraatz, Mareike, "Isolation of lactic acid-related bacteria from the pig mucosal proximal gastrointestinal tract, including *Olsenella umbonata* sp. nov. and *Veillonella magna* sp. nov.". Freien Universität Berlin, Dr. vet. med. dissertation, p. 55 (2011).
10. Näätsaari, Laura Hannele, "Horseradish peroxidase isoenzyme discovery and production in a new improved *Pichia pastoris* expression system". Graz University of Technology, Ph.D. dissertation, p. 125 (2012).
11. Ising, Christin, "The role of prohibitin-2 in podocytes – mitochondrial function and beyond". Universität zu Köln, Dr. rer. nat. dissertation, p. 18 (2014).
12. Hübner, Maria, "Development of Immunological Methods for the Detection of Micropollutants in Fresh Water Samples". Technische Universität München, Dr. rer. nat. dissertation, p. 120 (2015).
13. Hall, Diane Dogcio, "Standardisation and commercialisation of a filarial antibody test for use in the global lymphatic filariasis elimination programme". James Cook University, Ph.D. dissertation, p. 62 (2016).
14. Bosch, Belinda, "Molekulare Mechanismen der kardialen Zellschädigung nach experimentellem stumpfen Thoraxtrauma" ("Molecular mechanisms of cardiac cell damage after experimental blunt chest trauma"). Universität Ulm, Dr. med. dissertation, p. 31 (2018).
15. So, Michelle, "Analysis of C-peptide-specific CD4+ T cells in the peripheral blood of people with type 1 diabetes". University of Melbourne, Ph.D. dissertation, p. 103 (2018).
16. Findlay, Lucy, "Development of *in vitro* procedures that can better predict the safety of therapeutic monoclonal antibodies". University College London, Ph.D. dissertation, p. 284 (2019).
17. Binzager, Nada Omar, "The haematological factors and their disruption by organophosphorus compounds". Nottingham Trent University, Ph.D. dissertation, p. 48 (2019).
18. Chen, Peilei, "Analysis of two selected cell wall proteins and one lncRNA involved in desiccation tolerance of the resurrection plant *Craterostigma plantagineum*". Rheinischen Friedrich-Wilhelms-Universität Bonn, Dr. rer. nat. dissertation, p. 41 (2019).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

T2885pis Rev 03/22 CS,CMH,GCY,MAM

**MILLIPORE
SIGMA**