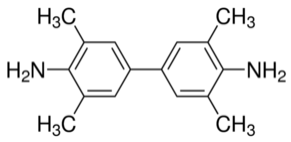
Product Information

# 3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Supersensitive, for ELISA

Ready-to-use solution

**T4444**

## 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 TMB-HRP reaction produces a soluble end-product that is blue in color and can be read spectrophotometrically at 370 nm or 650 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 that contains 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,[5-8] theses[9] and dissertations[10-20] have cited use of product T4444 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 or 2 N HCl, or of 0.5 M (1 N) $H_2SO_4$, 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 (Cat. No. S5814) is available for this application at 450 nm.
4. End-point assays can also be read at 650 nm using another Stop Reagent (Cat. No. S5689).
5. To reduce the intensity of a reaction, dilution of the antibodies or conjugates is suggested.

## References


1. Bos, E. *et al.*, *J. Immunoassay*, **2(3-4)**, 187-204 (1981).
2. Wróblewska, B. *et al.*, *Int. J. Food Sci. Tech.*, **39(8)**, 839-850 (2004).
3. Doig, N.M. *et al.*, *J. Neurosci.*, **30(44)**, 14610-14618 (2010).
4. Szymkiewicz, A., and Chudzik-Kozłowska, J., *Acta Alimentaria*, **43(2)**, 193-291 (2014).

5. Luo, Y. *et al*., *J. Lipid Res*., **50(8)**, 1581-1588 (2009).
6. Heussner, A.H. *et al*., *Toxins (Basel)*, **2(6)**, 1582-1594 (2010).
7. Carrion, J. *et al*., *J. Immunol*., **189(6)**, 3178-3187 (2012).
8. Piccoli, L. *et al*., *Nat. Commun*., **6**, 7375 (2015).
9. Manamela, Tebogo Sabina, "Isolation and characterization of immunoglobulin G from *Panthera leo* in South Africa and Zimbabwe". University of South Africa, M.Sc. thesis, p. 36 (2020).
10. Hämmerle, Monika, "Characterization of lymphatic vessels and lymphatic endothelial cells in type 2 diabetes mellitus". Medizinische Universität Wien, Ph.D. dissertation, p. 46 (2012).
11. Gnanapavan, Sharmilee, "Putative Biomarkers of Neuro-restoration in the CNS". University College London, Ph.D. dissertation, Appendix, p. ii (2013).
12. Sotiriou, Alexandros, "Novel targets for the development of drugs for Type 2 Diabetes Mellitus". Wageningen University, Ph.D. dissertation, pp. 79 (2016).
13. Gavrila, Adelina, "Modulating steroid insensitive pathways in airway smooth muscle cells". Leicester University, Ph.D. dissertation, p. 56 (2016).
14. Stinca, Sara, "Serum and dried blood spot thyroglobulin as a novel biomarker of iodine status in pregnancy". ETH Zürich, Dr. sc. dissertation, p. 53 (2016).
15. Alzahrani, Abdulrahman Muidh, "Airway Smooth Muscle and Mast Cell Interaction Modulates Corticosteroids Sensitivity". Leicester University, Ph.D. dissertation, p. 53 (2018).
16. Götzfried, Jessica Tanja Tamara, "Genetic, biochemical and preclinical studies on a tandem cluster of two human serpins: alpha-1-antitrypsin and serpina2". Ludwig-Maximilians-Universität München, Ph.D. dissertation, p. 34 (2018).
17. Kouhestani, Dina, "Complementation of a bimolecular Antibody-Derivative within the context of the Immunological Synapse". Julius-Maximilians-Universität Würzburg, Ph.D. dissertation, p. 31 (2019).
18. Wolfe, Michael, "Point-of-need biosensors for the detection of respiratory biomarkers". McMaster University, Ph.D. dissertation, p. 79 (2019).
19. Buist, Hanna Katrina, "Development of Isoform-Selective Affimers as Novel High Affinity Agents to Study EPAC1 Function". Heriot-Watt University, Ph.D. dissertation, p. 29 (2021).
20. Švab, Živa, "Identification and Molecular Characterization of Process Relevant MicroRNAs in Chinese Hamster Ovary (CHO) Cells: CHO Optimization for the Production of Recombinant Proteins of Pharmaceutical Interest". The Open University, Ph.D. dissertation, pp. 75, 76 (2021).

## 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.

### Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

### Contact Information

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



T4444dat Rev 03/23 AGW,GCY,MAM