

Product Information

3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for ELISA

T0440

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 or 655 nm. The reaction may be stopped with 2 M H₂SO₄, resulting in a yellow solution that is read at 450 nm.

This product is supplied as a ready-to-use peroxidase substrate containing TMB in a mildly acidic buffer. Prior to reaction with peroxidase, the substrate should be a colorless to light bluish-green solution. The substrate system develops a blue reaction product when reacted with peroxidase in microwell applications (such as ELISA assays). 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 references,⁵⁻¹⁸ theses¹⁹⁻²² and dissertations²³⁻³⁰ have cited use of T0440 in their research.

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.

Reagent

This substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Storage/Stability

Store this product at 2-8 °C.

Procedure

- This product is a ready-to-use, one-component substrate for peroxidase and is supplied at the working dilution.
- This reagent should be brought to room temperature (~25 °C) before use.
- Following reaction with peroxidase, a blue reaction product forms that may be read either at 370 nm, or between 620 nm 655 nm.
- For end-point assays, the reaction can be stopped by the addition of a volume of 1 N or 2 N HCl, or 1 N H₂SO₄, 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.
- Dilution of the substrate is **not** recommended. To reduce the intensity of a reaction, it is suggested that the antibodies or conjugates be diluted.

References

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- Bos, E. et al., J. Immunoassay, 2(3-4), 187-204 (1981).
- Wróblewska, B. et al., Int. J. Food Sci. Tech., 39(8), 839-850 (2004).
- 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. Black, W.C. et al., J. Med. Entomol., **43(2)**, 238-247 (2006).
- 6. Le Droumaguet, B., and, Velonia, K., *Angew. Chem. Int. Ed. Engl.*, **47(33)**, 6263-6266 (2008).
- 7. Rydell, G.E. *et al.*, *Glycobiology*, **19(3)**, 309-320 (2009).
- 8. Güven, A. *et al.*, *J. Nanobiotechnology*, **8**, 14 (2010).
- 9. Muhammad, N. et al., *J. Nanobiotechnology*, **9**, 8 (2011).



- Touboul, T. et al., "Directed Differentiation of Human Pluripotent Stem Cells into Fetal-Like Hepatocytes", in *Human Stem Cell Manual: A Laboratory Guide*, 2nd ed. (J. Loring, S. Peterson, eds.). Academic Press, Chapter 28, pp. 413-432 (2012).
- 11. Jouve, N. et al., J. Biol. Chem., **288(4)**, 2571-2579 (2013).
- 12. Hagbom, M. et al., Sci. Rep., 5, 11431 (2015).
- 13. Frei, J.C., and Lai, J.R., *Methods Enzymol.*, **580**, 45-87 (2016).
- 14. Gearhart, T.L. et al., J. Immunol. Methods, **435**, 85-89 (2016).
- 15. Thålin, C. et al., Immunol. Res., **65(3)**, 706-712 (2017).
- 16. Guerrero, M., et al., J. Sports Med. Phys. Fitness, **59(11)**, 1828-1834 (2019).
- 17. Honjo, K. et al., Cell Rep. Med., **2(1)**, 100164 (2020).
- 18. Shilts, J. et al., Sci. Rep., 11(1), 413 (2021).
- 19. Staren, Daniel M., "Characterization of the Coca Chemokine Receptor Four Agonist Activity of Ubiquitin". Loyola University Chicago, M.S. thesis, p. 15 (2012).
- 20. Sang, Sheila J., "The Use of Phage Display to Identify Specific Peptide Ligands". Youngstown State University, M.S. thesis, p. 19 (2014).
- 21. Nadhom, Hama, "Protein Microparticles for Printable Bioelectronics". Linköping University, M.S. thesis, pp. 22, 25, 69, 70 (2015).
- 22. Guo, Shenchung, "Bioprocess Development for Recombinant Therapeutic Protein Osteopontin from *Escherichia Coli*". Texas A&M University, M.S. thesis, p. 30 (2018).
- 23. Bhura, Dheeraj Kumar, "3D Interdigitated Electrode Array (IDEA) Biosensor For Detection Of Serum Biomarker". Portland State University, Ph.D. dissertation, p. 42 (2011).
- Matthews, Kerryn, "Immunological Analysis of Pericardial Tuberculosis". University of Cape Town, Ph.D. dissertation, p. 60 (2011).
- 25. Rondelli, Catherine Michelle, "XPC Haplotypes Alter DNA Repair Capacity and Levels of Genetic Damage". University of Texas Medical Branch Galveston, Ph.D. dissertation, p. 39 (2013).
- 26. Hauser, Barbara, "Mechanism of bone loss in rheumatic diseases". University of Edinburgh, Ph.D. dissertation, p. 88 (2015).

- 27. He, Peijun, "Development of paper-based point-of-care biosensors by laser-based direct-write processes". University of Southampton, Ph.D. dissertation, p. 113 (2017).
- 28. Liu, Lizhen, "The Functions of EP300 in Activated Pancreatic Stellate Cells and the Drug Resistance Problem in Pancreatic Cancer". Ruperto-Carola Universität Heidelberg, Dr. rer. nat. dissertation, p. 20 (2017).
- 29. Townsend, Catherine Louise, "Characterisation of Naïve and Antigen-Experienced Human Antibody Repertoires". King's College London, Ph.D. dissertation, pp. 110, 112, 117 (2018).
- 30. Reolizo, Lien Mari P., "Potential of Proline Rich Homeodomain (PRH) to inhibit intimal thickening". University of Bristol, Ph.D. dissertation, p. 46 (2021).

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