Sigma-Aldrich.

Product Information

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

T0440

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 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, $^{5-18}$ theses $^{19-22}$ and dissertations $^{23-30}$ 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 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.
- 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

- 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).
- Black, W.C. et al., J. Med. Entomol., 43(2), 238-247 (2006).
- Le Droumaguet, B., and, Velonia, K., Angew. Chem. Int. Ed. Engl., 47(33), 6263-6266 (2008).
- Rydell, G.E. *et al.*, *Glycobiology*, **19(3)**, 309-320 (2009).
- Güven, A. *et al.*, *J. Nanobiotechnology*, **8**, 14 (2010).



- 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).
- 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).
- 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).
- Nadhom, Hama, "Protein Microparticles for Printable Bioelectronics". Linköping University, M.S. thesis, pp. 22, 25, 69, 70 (2015).
- Guo, Shenchung, "Bioprocess Development for Recombinant Therapeutic Protein Osteopontin from *Escherichia Coli*". Texas A&M University, M.S. thesis, p. 30 (2018).
- 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).
- 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).
- Hauser, Barbara, "Mechanism of bone loss in rheumatic diseases". University of Edinburgh, Ph.D. dissertation, p. 88 (2015).

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

 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).

- 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).
- Townsend, Catherine Louise, "Characterisation of Naïve and Antigen-Experienced Human Antibody Repertoires". King's College London, Ph.D. dissertation, pp. 110, 112, 117 (2018).
- Reolizo, Lien Mari P., "Potential of Proline Rich Homeodomain (PRH) to inhibit intimal thickening". University of Bristol, Ph.D. dissertation, p. 46 (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 <u>SigmaAldrich.com/techservice</u>.

Standard Warranty

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

Contact Information

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

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.

