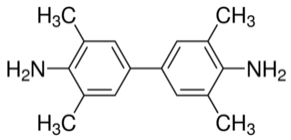
Product Information

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

Peroxidase substrate

T8665

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

Several references,[5-10] theses[11-13] and dissertations[14-24] have cited use of product T8665 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.

Reagent

This product combines soluble TMB chromogen, buffer, and hydrogen peroxide in a convenient, ready-to-use, single solution system.

Storage/Stability

Store this product at 2-8 °C. This product is light-sensitive and should be protected from direct sunlight or UV sources. Protect reagent from exposure to excess light and air by storing in the tightly sealed original container until immediately before use. Reseal container immediately after dispensing reagent.

Particulates may form and precipitate over time. Such particulates do not adversely affect the product integrity or product suitability.

Procedure

1. Allow reagent to come to room temperature prior to dispensing.
2. Use 200 µL of TMB solution per each microwell. No additional dilution or ingredients are required.
3. Incubate at room temperature for 30 minutes to develop the blue end product that can be read at 370 nm or 655 nm.
4. Acidification with 100 µL of a 0.5 M H_2SO_4 stopping solution, after the 30-minute incubation period, will produce a yellow color that can be read at 450 nm.

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. Cole, L.A. *et al*., *Clin. Chem*., **45(12)**, 2109-2119 (1999).
6. Figini, M., and Canevari, S., *Methods Mol. Biol*., **178**, 207-217 (2002).
7. Stepanchick, A. *et al*., *Cell. Physiol. Biochem*., **26(3)**, 363-374 (2010).
8. Combs, B. *et al*., *Methods Cell Biol*., **141**, 45-64 (2017).

9. Sypabekova, M. *et al*., *Tuberculosis*, **104**, 70-78 (2017).
10. Wong, M.K. *et al*., *PLoS ONE*, **13(6)**, e0199632 (2018).
11. Caffall, Zachary Farr, "Development of monoclonal and polyclonal antibodies against feline proinsulin and c-peptide". University of Georgia, M.S. thesis, p. 16 (2006).
12. Waldbrook, Matthew George, "*In vivo* efficacy of novel antibacterial and immunomodulatory synthetic peptides". University of British Columbia, M.Sc. thesis, p. 17 (2008).
13. Zhang, Jianchao, "HIV-1/SIV neutralizing antibody gene delivery: a novel vaccination approach". The Ohio State University, M.S. thesis, p. 80 (2009).
14. Jin, Zhaowei, "Microsphere formulation strategies, cell uptake studies, and pharmacokinetics in rats". Mercer University, Ph.D. dissertation, p. 95 (2004).
15. Thornton, Emma, "The Role of Substance P in early experimental Parkinson's Disease". University of Adelaide, Ph.D. dissertation, p. 78 (2008).
16. Wang, Kathryn Xiaozhao, "Osteopontin: Role in immune regulation and stress responses". Rutgers, The State University of New Jersey, Ph.D. dissertation, p. 62 (2008).
17. Pooley, Emma L., "Maternal inbreeding depression in the Zebra Finch, *Taeniopygia guttata*". University of Glasgow, Ph.D. dissertation, p. 34 (2013).
18. Thomas, Lian E., "Epidemiology of *Taenia solium* Cysticercosis in western Kenya". University of Edinburgh, Ph.D. dissertation, pp. 49, 128 (2013).
19. Holeiter, Monika, "Identification and production of cardio-inductive extracellular matrix proteins for applications in regenerative medicine". Universität Stuttgart, Dr. rer. nat. dissertation, p. 34 (2014).
20. Klaric, Goran, "Oral delivery of protein antigens to Atlantic salmon". University College London, Ph.D. dissertation, p. 65 (2016).
21. Raines, Lily, "Using Biochemical and Structural Approaches to Study EGFR-family Heterodimers". Johns Hopkins University, Ph.D. dissertation, p. 81 (2016).
22. Lubow, Jay, "HIV Infection of Macrophages Is Restricted by Mannose Receptor". University of Michigan, Ph.D. dissertation, p. 113 (2020).
23. Gerhards, Ramona Nadine, "Autoimmunity against oligodendrocyte myelin glycoprotein (OMGP)". Ludwig-Maximilians-Universität München, Dr. rer. nat. dissertation, p. 26 (2020).
24. Wong, Michael K., "The design and characterization of microphysiological platforms to model the human placenta". McMaster University, Ph.D. dissertation, p. 60 (2020).

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