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Product Information

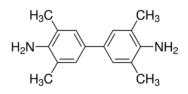
3,3',5,5'-Tetramethylbenzidine

Tablet, 1 mg substrate per tablet

T5525

Product Description

Storage Temperature: 2-8 °C Structure (TMB):



3,3',5,5'-Tetramethylbenzidine (TMB) is a colorimetric substrate that is used with horseradish 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 (H₂SO₄) and read at 450 nm.

Each T5525 tablet contains 1 mg of TMB substrate. T5525 is available in packages of 50 tablets or 100 tablets per box, individually foil wrapped for ease of use, storage, and safety.

Several theses⁶⁻⁸ and dissertations⁹⁻¹⁸ have cited use of T5525 in their research protocols.

Storage/Stability

Store the TMB tablets at 2-8 °C. Protect from heat, light, and moisture. Allow tablets to reach room temperature prior to use.

Preparation Instructions

TMB Substrate Solution

Option 1

- Dissolve one TMB tablet in 1 mL of DMSO.
- Add dissolved TMB solution to 9 mL of 0.05 M Phosphate-Citrate Buffer, pH 5.0.
- Add 2 µL of fresh 30% hydrogen peroxide per 10 mL of substrate buffer solution, immediately prior to use.

Option 2

- Dissolve one TMB tablet in 1 mL of DMSO.
- Add dissolved TMB solution to 9 mL of 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.03% sodium perborate (capsules, Cat. No. P4922).

Phosphate-Citrate Buffer Preparation

To prepare 0.05 M phosphate-citrate buffer, pH 5.0:

Option 1

• Dissolve one phosphate-citrate buffer tablet (such as Cat. No. P4809) in 100 mL of ultrapure water with stirring.

Option 2

- Mix 25.7 mL of 0.2 M dibasic sodium phosphate, 24.3 mL of 0.1 M citric acid, and 50 mL of ultrapure water.
- Adjust the pH to 5.0, if necessary.

Stop Solution

The reaction may be stopped by the addition of 50 μL of 2 M H_2SO_4 per 200 μL of reaction mixture.

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.

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).



- Rosenqvist, E. *et al.*, *Methods Mol. Med.*, **66**, 255-273 (2001).
- Slater, Nataliya, "In vitro selection of DNA aptamers that neutralise autoantibodies to cytosolic 5'-nucleotidase-1A". Murdoch University, B.Sc. honours thesis, p. 37 (2019).
- Pechlivanidis, Nikolaos G., "Towards Smartphone-Aided Electrochemical Detection for Point-of-Care Diagnostics". University of Southampton, M.Phil. thesis, p. 36 (2014).
- Anderson, Samantha Mary, "Characterisation of Fumonisin B1 toxicity in a cancerous liver cell line – Induction of Tissue Transglutaminase and the Endoplasmic Reticulum Stress Pathway". University of KwaZulu-Natal, M.Med.Sc. thesis, p. 37 (2013).
- Parthasarathy, Varadarajan, "The role of tetraspanins in multinucleated giant cell formation". University of Sheffield, Ph.D. dissertation, p. 59 (2005).
- Annan, Bernard Derek, "Optical Modulation of High-Affinity Biomolecules Function via Photochromic Dyes: A Step towards an Artificial Control of Biological Activity". Cranfield University, Ph.D. dissertation, p. 94 (2008).
- Castillo, Camilo Pohlenz, "Evaluation of Arginine and Glutamine as Dietary Supplements to Enhance *Edwardsiella Ictaluri* Vaccine Effectiveness in Channel Catfish". Texas A&M University, Ph.D. dissertation, p. 95 (2011).
- Kaneva, Magdalena Krasimirova, "Investigation into the immuno-therapeutic potential of melanocortin peptides on activated chondrocytes". University of Westminster, Ph.D. dissertation, p. 86 (2011).
- Zhang, Guopeng, "Protection of Lysozyme with Chitosan Using Radiant Energy Vacuum Dehydration for Control of Clostridial Necrotic Enteritis in Broiler Chickens". University of British Columbia, Ph.D. dissertation, p. 40 (2011).
- Benton, Donald James, "Biophysical Characterisation of the Involvement of Influenza A Virus Glycoproteins in Receptor Binding". University College London, Ph.D. dissertation, p. 84 (2015).
- Kumari, Vandana, "Mechanisms underlying the regulatory function of tumor necrosis factor-α in skin inflammation". Humboldt-Universität zu Berlin, Dr. rer. nat. dissertation, p. 33 (2015).

- Liu, Yifen, "Clinical Study on Apolipoprotein E Distribution, Metabolism and Glycation". University of Manchester, Ph.D. dissertation, p. 79 (2015).
- Myiang, Myint Zu, "Biological characterization of RNA polymerase proteins from human 2009 pandemic H1N1 and low pathogenic avian H5N2 and H9N2 influenza viruses". Nanyang Technological University, Singapore, Ph.D. dissertation, p. 57 (2016).
- Woo, Soo Ji, "Drug metabolism system associated with the exposure of benzo[a]pyrene and trichlorfon in rockfish (*Sebastes schlegelii*)". Pukyong National University, Ph.D. dissertation,, p. 14 (2019).

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