

Product Information

Stop Reagent for TMB Substrate

For ELISA, 450 nm

S5814

Product Description

In ELISA experiments which utilize horseradish peroxidase (HRP) conjugates and the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB), it is common practice to stop the reactions with dilute acid, such as HCl or H₂SO₄. This product is a proprietary, dry blend formulation that is used to stop the one-component TMB Liquid Substrate Systems (Cat. Nos. T5569, T4319, T8665, T0440, and T4444), where addition of this Stop Reagent changes the chromogen from blue to yellow.

Several publications,¹⁻⁸ theses,⁹ and dissertations¹⁰⁻¹⁶ cite use of S5814 in their protocols.

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

- Prior to hydration with water, this product is stable indefinitely.
- After hydration, the reagent is stable for a minimum of 2 years.
- Store at room temperature before and after reconstitution.
- Refrigerated temperatures will not harm the reagent.

Preparation Instructions

- Add 100 mL of distilled or deionized water.
- Allow approximately 5 minutes to dissolve.

Procedure

This product is used to stop TMB Microwell substrate and change the absorbance from blue (620 nm) to yellow (450 nm).

- Equal volumes of substrate and stop solution should be used.
- The resulting yellow end-product, which is stable for at least one hour, can then be read at 450 nm.
- Refer to the Product Information Sheets included with the respective TMB Liquid Substrate Systems for additional details.

References

1. Subramanian, R.R. *et al.*, *Nucleic Acids Res.*, **43(19)**, 9123-9132 (2015).
2. Levin, R.A. *et al.*, *Oncotarget*, **9(32)**, 22359-22367 (2018).
3. Liu, Y. *et al.*, *Front. Immunol.*, **9**, 1680 (2018).
4. Dunkley, J.C. *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **320(5)**, H1862-H1872 (2021).
5. Kim, J. *et al.*, *ACS Cent. Sci.*, **7(11)**, 1898-1907 (2021).
6. Maffei, M. *et al.*, *Biomolecules*, **11(12)**, 1812 (2021).
7. Wang, C. *et al.*, *Front. Pharmacol.*, **12**, 734603 (2021).
8. Dawson, E.D. *et al.*, *J. Immunol. Methods*, **504**, 113259 (2022).

9. Vélez, Crystal Alejandra Guluarte, "Efecto de ToxA y lisado de vibrio parahaemolyticus sobre el sistema inmune y morfología intestinal del huachinango del Pacífico (*Lutjanus peru*)" ["Effect of ToxA and lysate of vibrio parahaemolyticus on the immune system and intestinal morphology of Pacific huachinango (*Lutjanus peru*)"]. Centro de Investigaciones Biológicas del Noroeste, M.Sc. thesis, p. 41 (2016).
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. Pruvot, Mathieu, "Eco-epidemiology of production limiting diseases at the wildlife-livestock interface: beef cattle and elk in southwestern Alberta, Canada". University of Calgary, Ph.D. dissertation, p. 294 (2014).
12. Alsafi, Radi Taha, "Generation of Complex Recombinant Fowlpox Virus 9 (FP9) Encoding Simian Immunodeficiency Virus (SIVmac239) Sequences as a Model HIV Vaccine Candidate". University of Manchester, Ph.D. dissertation, p. 110 (2016).
13. Kamil, Atif, "Analysis of IgM sub-variants related to ancestral tetraploidy in salmonid fish". University of Bergen, Ph.D. dissertation, p. 73 (Appendix section) (2016).
14. Janssen-Duijghuijsen, Lonneke M, "Good, Bad, or Risky? Intestinal permeability to protein: Human and *in vitro* studies". Wageningen University, Ph.D. dissertation, p. 74 (2017).
15. Masheta, Dhafir Q., "Glutathione-decorated dendrons as potential drug carrier systems in multiple sclerosis". University of Brighton, Ph.D. dissertation, p. 138 (2017).

16. Petrou, Sabiha Hacibekiroglu, "Combined Cell and Gene Therapy Towards the Treatment of Age-related Macular Degeneration and Diabetic Retinopathy". University of Toronto, Ph.D. dissertation, p. 51 (2018).

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