

Product Information

BCIP®/NBT-Blue Liquid Substrate System for Membranes

Alkaline phosphatase substrate

B3804

Product Description

Storage temperature: 2-8 °C

5-bromo-4-chloro-3-indolyl phosphate (BCIP®) and nitro blue tetrazolium (NBT) are reagents that are widely used in tandem to detect alkaline phosphatase conjugates.¹⁻³

This BCIP®/NBT-Purple Liquid Substrate System for Membranes product is supplied as a ready-to-use buffered alkaline phosphatase substrate that contains both BCIP® and NBT. Prior to reaction with alkaline phosphatase, the substrate should appear as a colorless to light yellow solution. It will develop a permanent dark blue-purple reaction product when reacted with alkaline phosphatase on membrane applications (such as blotting).

Because this substrate produces an insoluble reaction product, this product **is not recommended** for multiwell applications (such as ELISA) or for immunohistochemical applications. Several publications,⁴⁻¹⁷ theses,¹⁸ and dissertations¹⁹⁻²¹ have cited use of product B3804 in their protocols.

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.

Preparation Instructions

The product is a ready-to-use, one component substrate for alkaline phosphatase and is supplied at the working dilution.

Storage/Stability

Store the product at 2-8 °C. This substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Usage

- The product should be brought to room temperature (~25 °C) before use.
- Use enough substrate solution to cover completely the membrane surface.
- Upon incubation, a dark blue-purple reaction product will develop on membrane sites where alkaline phosphatase is present.
- The reaction should be monitored continuously and read before the background color becomes too intense, which will result in diminished contrast between positive and background staining.
- Stop the reaction by rinsing briefly with water.
- **Dilution of the substrate product is not recommended.** To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted instead.

References

1. Blake, M.S. *et al.*, *Anal. Biochem.*, **136(1)**, 175-179 (1984).
2. Horowitz, J.P. *et al.*, *J. Med. Chem.*, **9(3)**, 447 (1966).
3. Smejkal, G.B., and Kaul, C.A., *J. Histochem. Cytochem.*, **49(9)**, 1189-1190 (2001).
4. Colca, J.R. *et al.*, *Am. J. Physiol. Endocrinol. Metab.*, **286(2)**, E252-E260 (2004).
5. Christiansen, S.P., and McLoon, L.K., *Invest. Ophthalmol. Vis. Sci.*, **47(2)**, 605-613 (2006).
6. Li, X. *et al.*, *Oncogene*, **25(4)**, 609-621 (2006).
7. Hearty, S. *et al.*, *J. Microbiol. Methods*, **66(2)**, 294-312 (2006).

8. Ehrlich, H. *et al.*, *Nat. Chem.*, **2(12)**, 1084-1088 (2010).
9. Katkar, P. *et al.*, *Metallomics*, **6(1)**, 117-125 (2014).
10. Ravichandran, E. *et al.*, *Pharm. Res.*, **33(3)**, 639-652 (2016).
11. Nashchekina, Yu.A. *et al.*, *Bull. Exp. Biol. Med.*, **163(1)**, 123-128 (2017).
12. Chiarella, E. *et al.*, *Int. J. Mol. Sci.*, **19(12)**, 4095 (2018).
13. Di Vito, A. *et al.*, *Cell Transplant.*, **28(1)**, 129-139 (2019).
14. Di Vito, A. *et al.*, *Cell Transplant.*, **29**, 963689720948497 (doi: 10.1177/0963689720948497) (2020).
15. Cha, Y. *et al.*, *Cell Rep.*, **37(13)**, 110155 (2021).
16. Liu, W. *et al.*, *Front. Neurol.*, **13**, 821792 (2022).
17. Rahantamalala, A. *et al.*, *PLoS Negl Trop Dis.*, **16(4)**, e0010265 (2022).
18. Routh, Melissa Anne, "SC-26196, A Delta-6 Desaturase Inhibitor, Normalizes Glucose Tolerance In ob/ob Mice". Colorado State University, M.S. thesis, p. 17 (2012).
19. Hegermann, Jan, "Untersuchungen von Cytoskelett-Komponenten und Motilität bei *Mycoplasma pneumoniae*" ("Studies of cytoskeletal components and motility in *Mycoplasma pneumoniae*"). Georg-August-Universität Göttingen, Dr. rer. nat. dissertation, p. 36 (2003).
20. Busin, Valentina, "The development of microfluidic paper-based analytical devices for point-of-care diagnosis of sheep scab". Heriot-Watt University, Ph.D. dissertation, p. 71 (2017).
21. Vyas, Rutesh Niranjnabhai, "Epigenetic Regulation of Olfactory Receptor Genes and Dedifferentiation of an Olfactory Placode Cell Line". Wesleyan University, Ph.D. dissertation, p. 224 (2019).

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B3804dat Rev 08/22 RBG,ALC,GCY,MAM

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