

Product Information

BCIP®/NBT-Purple Liquid Substrate System for Membranes

Ready-to-use alkaline phosphatase substrate

B3679

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 containing BCIP® and NBT. Prior to reaction with alkaline phosphatase, the substrate should appear as a colorless to light yellow solution. It will develop an insoluble dark 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 multi-well applications (such as ELISA) or for immunohistochemical applications. Several publications,⁴⁻⁹ theses¹⁰⁻¹³ and dissertations¹⁴⁻²⁰ have cited use of product B3679 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 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. van Herpen, T.W.J.M. *et al.*, *Ann. Bot.*, **102(3)**, 331-342 (2008).
5. Loughran, S.T. *et al.*, *Int. J. Cancer*, **129(12)**, 2787-2796 (2011).
6. Andersen, T.K. *et al.*, *J. Virol.*, **91(23)**, e01340-17 (2017).

7. Gudjonsson, A. *et al.*, *J. Immunol.*, **198(7)**, 2785-2795 (2017).
8. Wiśniewska-Chudy, E. *et al.*, *Oncol. Rep.*, **37(4)**, 2295-2307 (2017).
9. Ruf, J. *et al.*, *J. Cell. Mol. Med.*, **23(10)**, 6805-6811 (2019).
10. Watson, S.J. *et al.*, *eLife*, **10**, e60960 (2021).
11. Qi, H. *et al.*, *Vaccines (Basel)*, **10(3)**, 407 (2022).
12. Tero, Ben, "CpSA Protein-Protein Interactions in Group B *Streptococcus*". University of Maine, Honors thesis (Bachelor's degree), p. 26 (2019).
13. Griffiths, Andrea, "Assessing Exposure to *Cryptococcus gattii*". University of British Columbia, M.Sc. thesis, p. 31 (2006).
14. Matsumoto, Fabiana Santos, "Caracterização das proteínas do saco vitelínico de embriões bovinos *Bos indicus*" ("Characterization of yolk sac proteins from *Bos indicus* bovine embryos"). Universidade de São Paulo, M.Sc. thesis, p. 45 (2007).
15. Liang, Yanan, "Studies on human antigenic protein AVS 41kDa from *Aspergillus versicolor*". Carleton University, M.Sc. thesis, p. 26 (2008).
16. Loughran, Sinéad T., "Expression and role of the human anti-apoptotic *bfl-1* gene in Hodgkin's Lymphoma". Dublin City University, Ph.D. dissertation, p. 54 (2007).
17. van Herpen, Teun W.J.M., "Coeliac Disease Safe Gluten: The challenge to reduce toxicity while preserving wheat technological properties". Wageningen University, Ph.D. dissertation, p. 51 (2008).
18. Yu, Deqiang, "Application of membrane chromatography in bioprocessing". McMaster University, Ph.D. dissertation, p. 151 (2009).
19. Ray, Tathagat Dutta, "Novel Complement Blocking Antibodies Against Serogroup B *N. meningitidis*". University of Massachusetts Medical School, Ph.D. dissertation, pp. 70, 101 (2010).
20. Liu, Ran, "Role of Knr4 protein in *Saccharomyces cerevisiae* morphogenesis and sensitivity to Killer toxin K9: localization *versus* Phosphorylation". Université de Toulouse, Ph.D. dissertation, p. 73 (2015).
21. Andersen, Tor Kristian, "APC-targeted DNA vaccines against pandemic influenza". University of Oslo, Ph.D. dissertation, p. 21 (2017).
22. Rajaei, Atefeh, "The CPSA and PSR Proteins of *Streptococcus agalactiae* Provide a United Front to Protect Against the Host Immune System". University of Maine, Ph.D. dissertation, p. 31 (2022).

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 SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

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

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

MilliporeSigma, BCIP, 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.

© 2009-2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

B3679dat Rev 08/22 RBG,ALC,GCY,MAM

**MILLIPORE
SIGMA**