Product Information

5-Bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt

BioReagent, for molecular biology, powder, ≥99%

B6777

Product Description

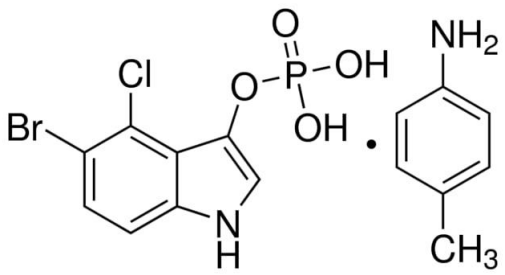
CAS Registry Number: 6578-06-9

Molecular Formula: $C_8H_6BrClNO_4P \cdot C_7H_9N$

Formula Weight: 433.62

Synonyms: BCIP® *p*-toluidine salt, X-phosphate *p*-toluidine salt

Storage temperature: –20 °C



5-Bromo-4-chloro-3-indolyl phosphate (BCIP®) and nitro blue tetrazolium (NBT) are commonly used in tandem for the colorimetric detection of alkaline phosphatase-labeled molecules.[1-3] The BCIP®/NBT substrate system is versatile and functions in a variety of applications, including:

- Northern, Southern, and Western blotting[4,5]
- in situ hybridization[6]
- immunohistochemistry[7]

BCIP® p-toluidine salt is soluble in dimethylformamide (DMF). DMF may be used to prepare a stock solution. A portion of the BCIP® stock solution is then combined with NBT in a reaction buffer to form a substrate solution for alkaline phosphatase. This substrate system, when incubated with alkaline phosphatase, produces an insoluble NBT diformazan product[8] that is easily observable with its purple color. (See Figure 1 for a reaction scheme.)

BCIP® is prepared synthetically. Cat. No. B6777 is tested for the absence of proteases, and thus is suitable for molecular biology applications. Several theses[9] and dissertations[10-21] have cited use of product B6777 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.

Solubility

BCIP® *p*-toluidine salt is tested for solubility in dimethylformamide (DMF) at 20 mg/mL.

Storage/Stability

Store BCIP® lyophilized product at −20 °C, protected from light and moisture.

A BCIP® stock solution in DMF remains active for ~2 weeks kept in the dark at 2-8 °C. However, a working solution in aqueous buffer is only good for one day. One publication has reported that 50 mg/mL stock solutions of BCIP in 100% DMF can be stored at −20 °C.[22]

References

1. Blake, M.S. *et al*., *Anal. Biochem*., **136(1)**, 175-179 (1984).
2. Green, F.J. (ed.), *The Sigma-Aldrich Handbook of Stains, Dyes & Indicators*, Aldrich Chemical Co., (Milwaukee, WI), p. 523 (1990).
3. Horowitz, J.P. *et al*., *J. Med. Chem*., **9(3),** 447 (1966).
4. Knecht, D.A., and Dimond, R.L., *Anal. Biochem*., **136(1)**, 180-184 (1984).
5. Leary, J.J., *et al*., *Proc. Natl. Acad. Sci. USA*, **80(13)**, 4045-4049 (1983).

6. Walters, C. *et al*., *J. Clin. Pathol*., **50(9)**, 749-754 (1997).

7. Meltzer, J.C. *et al*., *J. Histochem. Cytochem*., **45(4)**, 599-610 (1997).

8. McGadey, J., *Histochemie*, **23(2)**, 180-184 (1970).

9. Chang, Carolyn, "Understanding the Development and Growth of the Zebrafish (*Danio rerio*) Infraorbital Bones". Saint Mary's University, M.Sc. thesis, p. 34 (2013).

10. Altwein, Monika, "Lokalisierung und Funktion von Synaptischen Proteinen in der Netzhaut von Säugetieren" ("Localization and function of synaptic proteins in mammalian retina"). Technischen Universität Darmstadt, Dr. rer. nat. dissertation, p. 31 (2003).

11. Büttner, Angsar, "Vergleichende Untersuchungen über Translation und Transkription von Strukturproteinen des Felinen Leukämievirus nach experimenteller Infektion" ("Comparative studies on translation and transcription of feline leukemia virus structural proteins after experimental infection"). Justus-Liebig-Universität Gießen, Dr. med. vet. dissertation, pp. 200, 213 (2004).

12. Jacobsen, Björn, "Nachweis von *Mycoplasma bovis* in der Lunge von experimentell infizierten Kälbern mittels *in situ*-Hybridisierung" ("Detection of *Mycoplasma bovis* in the lungs of experimentally infected calves using *in situ* hybridization"). Tierärztliche Hochschule Hannover (University of Veterinary Medicine Hannover), Dr. med. vet. dissertation, p. 117 (2004).

13. Sihvo, Hanna-Kaisa, "Demonstration of Equine Interferon-γ mRNA, Transforming Growth Factor-β mRNA and Vascular Endothelial Growth Factor in Equine Idiopathic Focal Eosinophilic Enteritis". University of Helsinki, Dr. med. vet. dissertation, Appendix V, pp. 74, 75 (2008).

14. Lam, Win See, "Charakterisierung und immunologische Untersuchung von Seren von Sterilitätspatienten mit Entzündungszeichen im Genitaltrakt vor Initiation einer antientzündlichen Behandlung" ("Characterization and immunological analysis of sera from sterile patients with signs of inflammation in the genital tract prior to the initiation of anti-inflammatory treatment"). Rheinischen Friedrich-Wilhelms-Universität Bonn, Dr. rer. nat. dissertation, p. 21 (2015).

15. Jourdeuil, Karyn Allyson, "Deciphering the induction and patterning of the conjunctival papillae in the chicken, *Gallus gallus*". Dalhousie University, Ph.D. dissertation, p. 70 (2015).

16. Kumati, Osama, "Virus life cycle and the pathogenesis of malignant catarrhal fever". University of Nottingham, Ph.D. dissertation, p. 268 (2016).

17. Kupke, Alexandra, "Die Rolle des olfaktorischen Epithels in der initialen Phase der Infektion mit dem neurotropen *Borna disease virus*" ("The role of the olfactory epithelium in the initial phase of infection with the neurotropic *Borna disease virus*"). Justus-Liebig-Universität Gießen, Dr. med. vet. dissertation, p. 234 (2016).

18. Klingenstein, Moritz, "Charakterisierung von *in vitro* differenzierten olfaktorischen Rezeptorneuronen aus induziert pluripotenten Stammzellen" ("Characterization of *in vitro* differentiated olfactory receptor neurons from induced pluripotent stem cells"). Eberhard-Karls-Universität Tübingen, Dr. rer. nat. dissertation, p. 25 (2017).

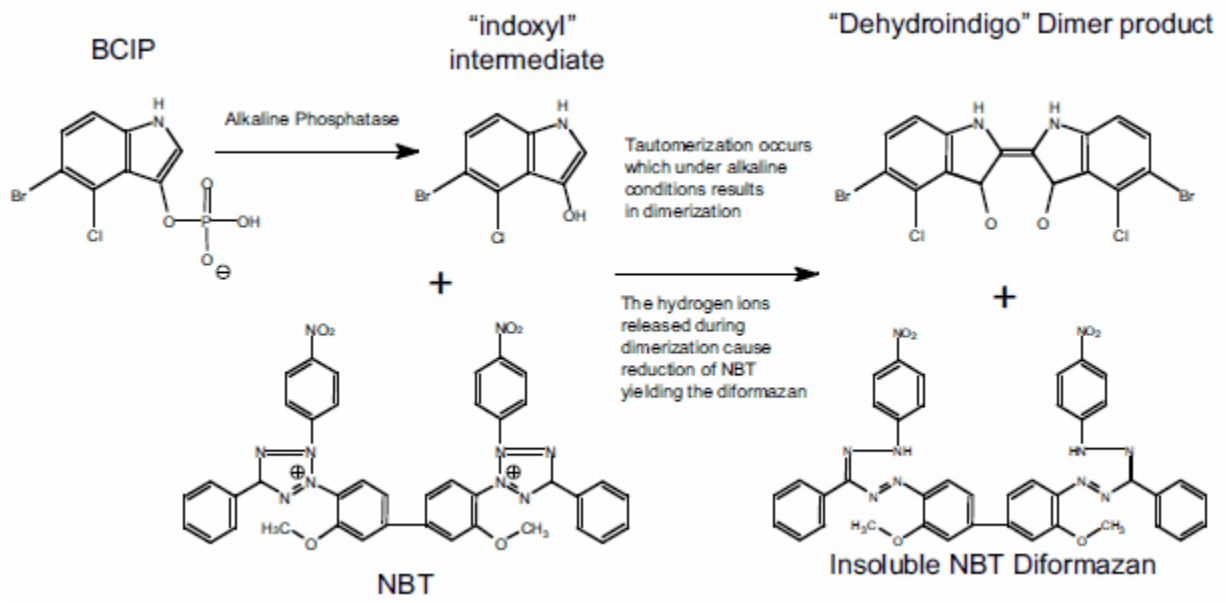
19. Raab, Stefanie, "Entwicklung und Charakterisierung eines iPSZ basierten Differenzierungsprotokolls zu olfaktorischen Rezeptorneuronen" ("Development and characterization of an iPSC based differentiation protocol to olfactory receptor neurons"). Eberhard-Karls-Universität Tübingen, Dr. rer. nat. dissertation, p. 28 (2017).

20. Tongtako, Witchaya, "*In situ* and *in vitro* characterization of canine and murine satellite glial cells and canine neurons from dorsal root ganglia". University of Veterinary Medicine Hannover, Dr. rer. nat. dissertation, p. 84 (2017).

21. Elias, Fernanda, "Caracterización y aplicaciones de un nuevo grupo de oligonucleótidos inmunoestimulantes en humanos" ("Characterization and use in humans of a new group of immunostimulatory oligonucleotides"). Universidad de Buenos Aires, Ph.D. dissertation, p. 49 (2018).

22. Byrne, C., and Hardman, M.J., *Methods Mol. Biol*., **289**, 127-136 (2005).

Figure 1. BCIP®/NBT Reactions



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.

B6777pis Rev 08/22 RBG,KTA,GCY,MAM