Product Information

5-Bromo-4-chloro-3-indolyl phosphate disodium salt

B6149

Product Description

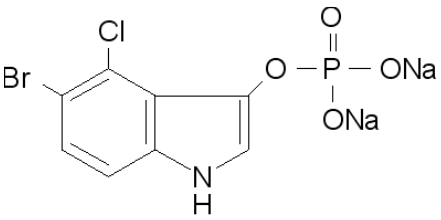
CAS Registry Number: 102185-33-1

Synonyms: BCIP® disodium salt, X-phosphate disodium salt

Molecular Formula: $C_8H_4BrClNO_4P \bullet 2Na$

Formula Weight: 370.43

Structure:



5-Bromo-4-chloro-3-indolyl phosphate (BCIP®) and nitro blue tetrazolium (NBT) are commonly used for colorimetric detection of alkaline phosphatase-labeled molecules. The BCIP®/NBT substrate system is versatile and functions in various applications, including Northern, Southern, and Western blotting, *in situ* hybridization, and immunohistochemistry.[1-8]

BCIP® disodium salt is soluble in water. A BCIP® disodium salt stock solution may be used in combination with NBT and a reaction buffer to form a substrate solution for alkaline phosphatase. When incubated with alkaline phosphatase, this substrate system produces an insoluble NBT diformazan product that is easily observable with its purple color, per the general reaction scheme in Figure 1.

BCIP® disodium salt is prepared synthetically. Several publications,[9-17] theses,[18] and dissertations[19-24] have cited use of product B6149 in their research 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

BCIP® disodium salt is soluble in water at 50 mg/mL. It is insoluble in DMF.

Storage/Stability

Store BCIP® disodium salt at –20 °C, protected from light and moisture.

Procedure

Sample Protocol for NBT/BCIP® System for Detection of Alkaline Phosphatase

This protocol may be used for such applications as Western blotting.

1. Prepare Substrate Buffer (0.1 M Tris, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5). Adjust pH with HCl.
2. Prepare NBT (Cat. No. N6639) stock solution at 10 mg/mL in water.
3. Prepare BCIP® disodium salt stock solution at 50 mg/mL in water.
4. Prepare BCIP®/NBT Substrate Solution by adding 33 µL of 50 mg/mL BCIP® disodium salt stock solution and 330 µL of 10 mg/mL NBT stock solution to 10 mL of Substrate Buffer.
5. Rinse specimens incubated with an alkaline phosphatase conjugate in a wash buffer (**non-phosphate**) before treatment with the BCIP®/NBT Substrate Solution. Cover the entire specimen with the reagent during color development.
6. Incubate the specimen at room temperature with the BCIP®/NBT Substrate Solution for ~10 minutes. Specimens and procedure may affect the length of time needed for color development.
7. Monitor color development to avoid over-development. Stop color development by rinsing the specimen with distilled water.

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. Horwitz, 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. McGadey, J., *Histochemie*, **23(2)**, 180-184 (1970).
7. Meltzer, J.C. *et al*., *J. Histochem. Cytochem*., **45(4)**, 599-610 (1997).
8. Walters, C. *et al*., *J. Clin. Pathol*., **50(9)**, 749-754 (1997).
9. Høyer, P.E., and Kirkeby, S., *J. Histochem. Cytochem*., **44(8)**, 855-863 (1996).
10. Pagnussat, G.C. *et al*., *Physiologia Plantarum*, **108(4)**, 337-344 (2000).
11. Weglarz, T.C., and Sandgren, E.P., *Proc. Nat. Acad. Sci. USA*, **97(23)**, 12595-12600 (2000).
12. Hagemann, T.L. *et al*., *J. Neurosci*., **26(43)**, 11162-11173 (2006).
13. Schreiner, P., *J. Exp. Microbiol. Immunol*., **13**, 93-99 (2009).
14. Qi, M. *et al*., *J. Bacteriol.*, **193(10)**, 2498-2509 (2011).
15. Wu, X. *et al*., *BMC Microbiol*., **11**, 87 (2011).
16. Cui, Z.-K. *et al*., *Nat. Commun*., **10(1)**, 3523 (2019).
17. Schepler, H. *et al*., *Theranostics*, **12(1)**, 18-34 (2022).
18. Antonakou, Vasiliki, "Lhx6 controls cIN development on a stage and IN-subtype specific manner". University of Athens, M.Sc. thesis, p. 14 (2019).
19. McKeand, Jacqueine B., "Aspects of the immunobiology of *Dictyocaulus viviparus* infection". University of Glasgow, Ph.D. dissertation, p. 50 (1992).
20. Sasselli, Valentina, "The potential role of Rac signalling and the Planar Cell Polarity pathway in wiring of the Enteric Nervous System". University College London, Ph.D. dissertation, p. 95 (2011).
21. Hamilton, Andrew, "Determination of the Structural Requirements for Modification of Vascular Endothelial Growth Factor Angiogenic Activity by Heparan Sulfate Oligosaccharides". University of Manchester, Ph.D. dissertation, pp. 79, 216 (2011).
22. Kalaitzidou, Melania, "The role of SATB1 in medial ganglionic eminence-derived cortical interneuron differentiation". University College London, Ph.D. dissertation, p. 99 (2014).
23. Velasco, Vera Marjorie Elauria, "Traits underlying phosphorus use by the extremophyte *Eutrema salsugineum*". McMaster University, Ph.D. dissertation, p. 41 (2017).
24. Matheus, Friederike, "The role of *Additional sex combs-like* genes in human pluripotent stem cell differentiation and congenital disorders". Ludwig-Maximilians-Universität zu München, Dr. rer. nat. Dissertation, p. 59 (2018).

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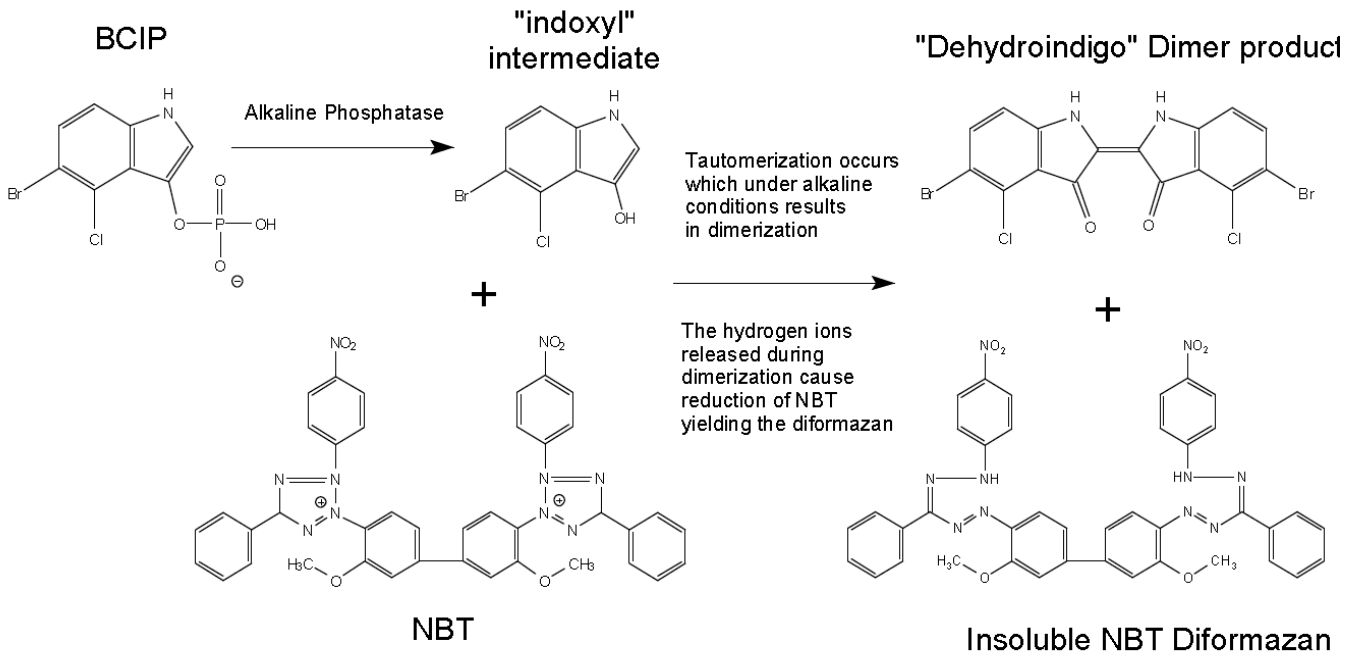
Figure 1. BCIP®/NBT Reactions



Troubleshooting Guide for Western Blotting

Problem	Suggestion
The background is too high.	Use a blocking step prior to application of the primary antibody. Normal serum (10% v/v) from the same species as the second antibody generally produces the best results.
	• Additional blocking agents for immunoblotting are 10% BSA, 0.05% TWEEN® 20, or 3% non-fat dried milk. • **Note**: Do not use milk as a blocking agent when using avidin-biotin systems.
	Decrease staining time.
	Titer the conjugate to optimize working dilution
No color develops or the color is too faint.	Adjust the concentration of the primary antibody
	Adjust the concentration of the secondary antibody
	Determine if the enzyme conjugate is active.
	Consider using an amplifying system such as avidin-biotin.
	Increase the staining time.
	Adjust the transfer time of the samples to the nitrocellulose membrane
	Increase the amount of sample.

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