

Product Information

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

Catalog Numbers **B1026** and **B6149**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

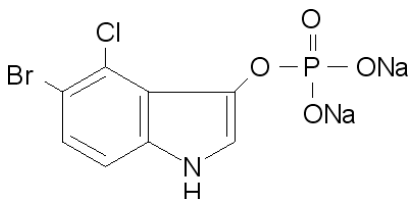
CAS RN 102185-33-1

Synonyms: BCIP disodium salt, X-phosphate disodium salt

Product Description

Molecular Formula: $\text{C}_8\text{H}_4\text{BrClNO}_4\text{P} \cdot 2\text{Na}$

Formula Weight: 370.43



BCIP is prepared synthetically. 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) are commonly used for the colorimetric detection of alkaline phosphatase-labeled molecules. The BCIP/NBT substrate system is versatile and functions in a variety of applications, including Northern, Southern, and Western blotting, *in situ* hybridization, and immunohistochemistry. BCIP disodium salt is soluble in water. It may be used to prepare a stock solution, which in combination with NBT and a reaction buffer, form a substrate solution for alkaline phosphatase. This substrate system, when incubated with alkaline phosphatase, produces an insoluble NBT diformazan product that is easily observable with its purple color (see Figure 1).

Product B1026 is a molecular biology reagent with no protease detected.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

BCIP disodium salt is soluble in water at 50 mg/ml and insoluble in DMF.

Storage/Stability

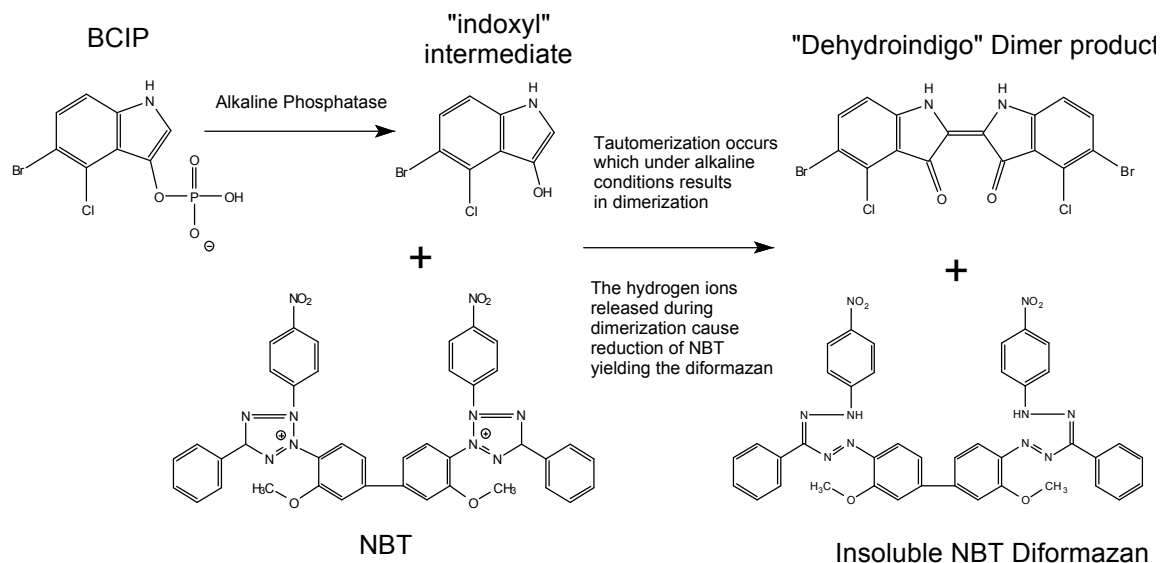
Store BCIP at $-20\text{ }^{\circ}\text{C}$, protected from light and moisture. It remains active of three years.

Procedure

The NBT/BCIP System for Detection of Alkaline Phosphatase – The standard protocol for Western blotting is as follows:

1. Prepare Substrate Buffer – 0.1 M Tris, 100 mM sodium chloride, and 5 mM MgCl_2 , pH 9.5. Adjust pH with HCl.
2. Prepare NBT, Catalog Number N6639, Stock Solution at 10 mg/ml in water.
3. Prepare BCIP disodium salt, Catalog Numbers B1026 or B6149, Stock Solution at 50 mg/ml in water.
4. Prepare BCIP/NBT Substrate Solution by adding 33 μl of 50 mg/ml BCIP 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.

Figure 1.
BCIP/NBT Reactions



Troubleshooting Guide for Western Blotting:

Problem	Suggestion
The background is too high.	Use a blocking step prior to the 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 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.	

References

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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., Substrates for Cytochemical demonstration of enzyme activity. II. Some Dihalo-3-indolyl phosphates and sulfates. *J. Med. Chem.*, **9**, 447 (1966).
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5. Leary, J.J., et al., Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: Bio-blots. *Proc. Natl. Acad. Sci. USA*, **80**, 4045-4049 (1983).
6. McGadey, J., A tetrazolium method for non-specific alkaline phosphatase. *Histochemie*, **23**, 180-184 (1970).
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8. Walters, C., et al., Detection of parvovirus B19 in macerated fetal tissue using *in situ* hybridization. *J. Clin. Pathol.*, **50**, 749-754 (1997).

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RBG,KTA,MAM 01/09-1

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