3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

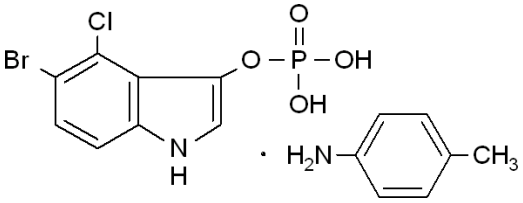
5-Bromo-4-chloro-3-indolyl phosphate
***p*-toluidine salt**

Catalog Numbers **B6777 and B8503**
Storage Temperature –20 °C

CAS RN 6578-06-9
Synonyms: BCIP® *p*-toluidine salt, X-phosphate *p*-toluidine salt

Product Description

Molecular Formula: $C_8H_6BrClNO_4P \cdot C_7H_9N$
Formula Weight: 433.62



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 p-toluidine salt is soluble in dimethylformamide. 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 B6777 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 p-toluidine salt is soluble in DMF at 20 mg/ml and insoluble in water.

Storage/Stability

Store BCIP at –20 °C, protected from light and moisture. It remains active of three years.

A BCIP stock in DMF remains active for ~2 weeks kept in the dark at 2–8 °C, but a working solution in aqueous buffer is only good for one day.

References

1. Blake, M.S., A rapid, sensitive method for detection of alkaline phosphatase-conjugated antibody on Western blots. Anal. Biochem., **136**, 175 (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., Substrates for Cytochemical demonstration of enzyme activity. II. Some Dihalo-3-indolyl phosphates and sulfates. J. Med. Chem., **9**, 447 (1966).
4. Knecht, D.A., and Dimond, R.L., Visualization of antigenic proteins on Western blots. Anal. Biochem., **136**, 180-184 (1984).
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).
7. Meltzer, J.C., et al., Enhanced immuno-histochemical detection of autonomic nerve fibers, cytokines and inducible nitric oxide synthase by light and fluorescent microscopy in rat spleen. J. Histochem. Cytochem., **45**, 599-610 (1997).
8. Walters, C., et al., Detection of parvovirus B19 in macerated fetal tissue using *in situ* hybridization. J. Clin. Pathol., **50**, 749-754 (1997).

Figure 1.
BCIP/NBT Reactions