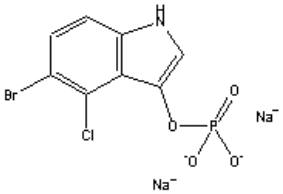
Catalog Number: 100368, 150042, 193989, 193991

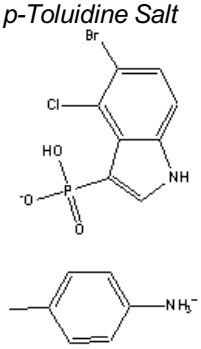
5-Bromo-4-chloro-3-indolylphosphate

Structure:

Sodium Salt



p-Toluidine Salt



Molecular Formula: $C_8H_4BrClNO_4PNa_2$
Molecular Weight: 370.4
CAS # : 102185-33-1

Molecular Formula: $C_8H_6BrClNO_4P \cdot C_7H_9N$
Molecular Weight: 433.7
CAS # : 6578-06-9

Synonyms: BCIP; X-phosphate

Physical Description: White to off-white powder

Purity: ≥98%

Description: A chromogenic substrate for alkaline phosphatase.

Typical Procedure for the Detection of Alkaline Phosphatase using a NBT/BCIP System:

Nitro Blue Tetrazolium (NBT) is used with the alkaline phosphatase substrate BCIP in immunostaining[1] and immunohistological[4] staining procedures. This substrate system produces an insoluble NBT diformazan end product that is blue in color and can be observed visually.

During the reaction tautomerization of the BCIP occurs, which under alkaline conditions, results in dimerization of the BCIP. The hydrogen ions released during dimerization cause reduction of NBT yielding the insoluble NBT diformazan product.

Use a substrate buffer consisting of 0.1 M Tris, 100 mM sodium chloride, 5 mM $MgCl_2$, pH 9.5, adjust the pH with HCl.

Add 33 ul of a 50 mg/ml stock solution of BCIP in water and 330 ul of a 10 mg/ml NBT stock solution in water to 10 ml of substrate buffer.

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. Incubate the specimen at room temperature with theh BCIP/NBT reagent for approximately 10 minutes. Specimens and procedure may affect the length of time needed for color development. Monitor the color development to avoid over-development. Stop the color development by rinsing the specimen with distilled water.

Troubleshooting the procedure:

1. Background is too high:

    a. 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.
    b. Additional blocking agents for immunoblotting are 10% BSA, 0.05% Tween 20, or 3% Non-fat dried milk powder; however, do not use the dried milk when using an avidin-biotin system.
    c. Decrease staining time.
    d. Titer the conjugate to optimize working dilution.

2. No color develops or color is too faint:

    a. Adjust the concentration of the primary antibody.
    b. Adjust the concentration of the secondary antibody.
    c. Determine if the enzyme conjugate is active.
    d. Consider using an amplifying system such as avidin-biotin.
    e. Increase the staining time.
    f. Adjust the transfer time of the samples to the nitrocellulose membrane.

g. Increase the amount of sample.

Solubility: Disodium salt is soluble in water (50 mg/ml); insoluble in dimethylformamide. The p-Toluidine salt is soluble in DMF (20 mg/ml) but insoluble in water. The most common buffer to use with BCIP and alkaline phosphatase is 0.1 M Tris, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5. Solutions should be prepared fresh for each use.

Solubility (of toluidine salt): Soluble in 1 N Sodium Hydroxide (0.5% w/v); DMF (1%-clear, slightly yellowish solution); insoluble in water

Availability:

Catalog Number	Description	Size
150042	5-Bromo-4-chloro-3-indolyl phosphate, disodium salt	50 mg 100 mg 250 mg 1 g
193989	5-Bromo-4-chloro-3-indolyl phosphate, disodium salt, molecular biology reagent	100 mg 500 mg
100368	5-Bromo-4-chloro-3-indolyl phosphate, p-Toluidine salt	25 mg 50 mg 100 mg 1 g
193991	5-Bromo-4-chloro-3-indolyl phosphate, p-Toluidine salt, molecular biology reagent	25 mg 100 mg 500 mg

Also Available:

Catalog Number	Description	Size
980781 980782	BCIP, stabilized substrate, ready-to-use; concentration 2.31 mmol/liter	100 ml 500 ml
100416	p-Nitro Blue Tetrazolium Chloride	50 mg 100 mg 250 mg 500 mg 1 g 5 g
193999	p-Nitro Blue Tetrazolium Chloride, molecular biology reagent	50 mg 250 mg 1 g
980791 980792	p-Nitro Blue Tetrazolium Liquid Substrate, stabilized substrate solution, ready-to-use; concentration 0.489 mmol/liter	100 ml 500 ml

References:

1. Blake, M.S., *Anal. Biochem.*, **v. 136**, 175 (1984).
2. Ey, P.L. and Ashman, L.K., *Meths. Enzymol.*, **v. 121**, 497 (1986).
3. Fikrig, S.M., et al., *The Lancet*, **Sept. 7**, p. 532 (1968).
4. Horowitz, J.P., et al., *J. Med. Chem.*, **v. 9**, 447 (1966).
5. Knecht, D.A. and Dimond, R.L., "Visualization of antigenic proteins on Western blots." *Anal. Biochem.*, **v. 136:1**, 180-184 (1984).
6. Leary, J.J., et al., "Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: Bioblots." *Proc. Natl. Acad. Sci.*, **v. 80**, 4045-4049 (1983).
7. McGadey, J., "A tetrazolium method for non-specific alkaline phosphatase." *Histochemie.*, **v. 23**, 180-184 (1970).
8. Meltzer, J.C., et al., "Enhanced immunohistochemical detection of autonomic nerve fibers, cytokines and inducible nitric oxide synthase by light and fluorescent microscopy in rat spleen." *J. Histochem. Cytochem.*, **v. 45:4**, 599-610 (1997).
9. Walters, C., et al., "Detection of parvovirus B19 in macerated fetal tissue using in situ hybridisation." *J. Clin. Pathol.*, **v. 50:9**, 749-754 (1997).