

Technical Data Sheet

BCIP (5-Bromo-4-chloro-3-indolyl phosphate p-toluidine salt)

for molecular biology

Order number: 1265

The artificial substrate BCIP is used for detection and visualisation of the alkaline phosphatase in various biochemical, molecular biological and immunohistochemical methods.

Dephosphorylation of BCIP in the presence of atmospheric oxygen results in formation of the deep blue dye 5,5'-dibromo-4,4'-dichloro-indigo that can easily be detected on tissue and blotting membranes.

Since BCIP itself is almost insoluble, 5-bromo-4-chloro-3-indolyl phosphate is usually used in the form of p-toluidine salt (such as this article 1265, soluble in DMF at 50 mg/ml) or sometimes as disodium salt (soluble in water at 20 mg/ml).

Application

BCIP serves as a chromogenic substrate in Northern, Southern and Western blotting, as well as in situ hybridization and immunohistochemistry.

BCIP (in combination with the redox dye NBT) sensitively and reliably indicates the presence of the enzyme. Alkaline phosphatase which is used as a selective marker for specific proteins or other targets. The antibody-coupled alkaline phosphatase catalyzes dephosphorylation of BCIP to 5-bromo-4-chloroindolyl, which dimerizes in air to a blue indigo dye. This reaction is amplified and accelerated by NBT, which is itself reduced to a blue di-formazan dye as a result of the reaction. The result is an insoluble, violet-colored precipitate that can be easily detected on membranes or tissue sections.

Application note for alkaline phosphatase detection with BCIP-p-toluidine salt and NBT in immunoblot procedures:

Stock solutions (protect from light and moisture and store at 2-8 °C)

- x 0.5 g NBT (article 1267) in 10 ml 70 % dimethylformamide
- x 0.5 g BCIP -p-toluidine salt in 10 ml 100 % dimethylformamide (article LC-4207)

Preparation of fresh substrate solution:

- x Add 66 µl of NBT stock solution to 10 ml of incubation buffer (100 mM NaCl, 5 mM MgCl₂, 100 mM Tris, pH 9.5)
- x Mix well
- x Add 33 µl of BCIP stock solution
- x Consume within one hour

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