# Technical Data Sheet

## NBT (Nitro Blue Tetrazolium)

**for molecular biology**

**Order number: 1267**

The redox dye NBT (nitro blue tetrazolium chloride) is used as a detection reagent in various biochemical, molecular biological and immunohistochemical methods.
The molecular reaction is always as follows: Reduction of the di-tetrazolium salt initially produces a red mono-formazan dye, which on further reduction changes to a blue di-formazan dye.
The NBT can either function as a direct enzyme substrate of various oxidoreductases and dehydrogenases, or it can be used as a colour enhancer in reactions of alkaline phosphatase and BCIP.

### Application

NBT serves as a chromogenic reagent in Northern, Southern and Western blotting, as well as in in situ hybridization and immunohistochemistry. NBT can also be used as a marker for various NADH- or NADPH-dependent reactions and for the determination of radical oxygen species.
The most common qualitative use of NBT is in the detection of alkaline phosphatase activity. Here, the combination of NBT and BCIP sensitively and reliably indicates the presence of the enzyme. Alkaline phosphatase can be used as a selective marker for specific proteins or other targets by coupling to an antibody. The antibody-coupled alkaline phosphatase dephosphorylates the artificial substrate BCIP to 5-bromo-4-chloroindolyl, which dimerizes in air to a blue 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)

- 0.5 g NBT in 10 ml 70 % dimethylformamide
- 0.5 g BCIP -p-toluidine salt (article 1265) in 10 ml 100 % dimethylformamide (article LC-4207)

Preparation of fresh substrate solution:

- 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)
- Mix well
- Add 33 µl of BCIP stock solution
- Consume within one hour

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