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NITRO-BLUE TETRAZOLIUM CHLORIDE

CAS Number: 298-83-9

Storage Temperature: 2-8℃

Product Number: N8140

Product Description :

Appearance: Yellow crystalline powder

Molecular formula: C40H30N10O6Cl2

Molecular weight: 817.6

NBT is prepared synthetically.The most common application for NBT is the detection of alkaline phosphatase on western blots. NBT has also been used as a redox indicator for other enzymatic reactions including dehydrogenases, threonine deaminase, glucose-6-phosphate dehydrogenase, phosphofructokinase on polyacrylamide gels, oxidases on polyacrylamide gels,and pentose shunt dehydrogenses. Redox and halfwave potentials have been determined for NBT. NBT has also been used as a colorimetric indicator of bacterial infection in blood samples.

Preparation Instructions:

NBT is soluble in H2O at 10 mg/ml, ethanol at 5 mg/ml and 2-methoxyethanol at 20 mg/ml. A stock solution at 10 mg/ml in water is stable 1-2 weeks in the dark at 2-8 °C.

Storage/Stability:

NBT has a shelf life of three years when stored at 2-8 °C and protected from light.

Procedure :

The NBT/BCIP System for Detection of Alkaline Phosphatase

Nitro Blue Tetrazolium (NBT) is used with the alkaline phosphatase substrate 5 Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) in immunoblotting and immunohistological staining procedures. This substrate system produces an insoluble NBT diformazan end product that is blue in color and can be observed visually.

The standard protocol for western blotting is as follows:

1. Prepare substrate buffer: 0.1 M Tris, 100 mM sodium chloride, 5 mM MgCl2, pH 9.5, adjust pH with HCl.
2. Prepare NBT stock solution at 10 mg/ml in water.
3. Prepare BCIP disodium salt stock solution at 50 mg/ml in water. BCIP p-toluidine salt is soluble in DMF and insoluble in water.
4. 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.
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 reagent for approximately 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.