

NBT

34035

0154.2

Number	Description
34035	NBT (nitro-blue tetrazolium chloride), 1g

Storage: Upon receipt store product at 4°C protected from moisture. Product is shipped at ambient temperature.

Introduction

Thermo Scientific NBT (nitro-blue tetrazolium chloride) yields a blue-purple, water-insoluble product upon reduction of the tetrazolium salt. The product of reduction is NBT-formazan. This substrate is widely used for immunochemistry applications because the color is linear and stable. NBT is used as the substrate for both glucose oxidase and alkaline phosphatase systems.

NBT Substrate Solution for Glucose Oxidase Applications

- NBT Stock Solution: Dissolve 0.5g NBT in 10mL of 70% dimethylformamide in water. This stock solution is stable at 4°C for ~1 year.
 - Substrate Solution: 41.7mM (7.5g/L) α -D-glucose, 326 μ M (0.1g/L) PMS (phenazine methosulfate) in 0.1M Na₂HPO₄, pH 6.9.
1. Add NBT stock solution to the substrate solution at a final concentration of 2.45mM (0.5g/L).
 2. Mix and store in the dark at 4°C for 1 hour before use to allow muta-rotation of α -D-glucose to β -D-glucose.
 3. Filter solution through a 0.22 μ m filter before use to remove precipitated salts.

Note: PMS is light sensitive and can reduce the tetrazolium salt during storage. Prepare the substrate solution immediately before use.

NBT/BCIP Substrate Solution for Alkaline Phosphatase Applications

- NBT Stock Solution: Dissolve 0.5g of NBT in 10mL 70% dimethylformamide (DMF) in water. This stock solution is stable at 4°C for ~1 year.
- BCIP (Product No. 34040) Stock Solution: Dissolve 0.5g of BCIP in 10mL 100% DMF. This stock solution is stable at 4°C for ~1 year.
- Substrate Buffer: 100mM Tris, pH 9.5, containing 100mM NaCl and 5mM MgCl₂.
- Substrate Solution: Add 66 μ L of stock NBT to 10mL of Substrate Buffer and mix well. Add 33 μ L of stock BCIP to the NBT/Substrate Buffer solution. Use this solution within 1 hour.

Related Thermo Scientific Products

34040	BCIP (5-bromo-4-chloro-3'-indolylphosphate <i>p</i> -toluidine salt), 1g
34042	1-Step™ NBT/BCIP, 250mL
31872	Normal Goat Serum, 2mL
31884	Normal Rabbit Serum, 2mL
32040	ABC Standard Alkaline Phosphatase Staining Kit
31002	NeutrAvidin® Protein, Alkaline Phosphatase Conjugated, 2mg
21323	Streptavidin, Alkaline Phosphatase Conjugated, 3mg

General References

- Altman, F.P. (1976). Tetrazolium salts and formazans. *Progr Histochem Cytochem* **9**:1-56.
- Fink, B., *et al.* (1979). Demonstration of viral antigen in cryostat sections by a new immunoperoxidase procedure eliminating endogenous peroxidase activity. *J Histochem Cytochem* **27**:686-8.
- Nakamura, S. and Ogura, V. (1968). Mode of inhibition of glucose oxidase by metal ions. *J Biochem* **64**:439-47.
- O'Malley, J.J. and Weaver, J.L. (1972). Subunit structure of glucose oxidase from *Aspergillus niger*. *Biochem* **11**:3527-32.
- Straus, W. (1979). Peroxidase procedures. Technical problems encountered during their application. *J Histochem Cytochem* **27**:1349-51.

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