

# 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)  $\alpha$ -D-glucose, 326 $\mu$ M (0.1g/L) PMS (phenazine methosulfate) in 0.1M Na<sub>2</sub>HPO<sub>4</sub>, 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  $\alpha$ -D-glucose to  $\beta$ -D-glucose.
  3. Filter solution through a 0.22 $\mu$ 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 MgCl<sub>2</sub>.
- Substrate Solution: Add 66 $\mu$ L of stock NBT to 10mL of Substrate Buffer and mix well. Add 33 $\mu$ L of stock BCIP to the NBT/Substrate Buffer solution. Use this solution within 1 hour.

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## Related Thermo Scientific Products

|       |   |
|-------|---|
| 34040 | BCIP (5-bromo-4-chloro-3'-indolylphosphate <i>p</i> -toluidine salt), 1 g |
| 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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