

# 1-Step™ NBT/BCIP plus Suppressor

**34070**

0561.2

Number	Description
34070	1-Step NBT/BCIP plus Suppressor, 100mL of substrate containing 1mM levamisole

**Storage:** Upon receipt store at 4°C. Product is shipped with an ice pack.

## Introduction

The Thermo Scientific 1-Step NBT/BCIP plus Suppressor is a one-component alkaline phosphatase substrate that inhibits endogenous phosphatase activity. This substrate reacts with calf intestinal alkaline phosphatase to produce a dark purple precipitate and is ideal for immunohistochemistry and *in situ* hybridization applications. Two forms of alkaline phosphatase exist in mammals: one form is distributed in a variety of tissues such as the kidney, liver, placenta, bone and tumor; the other form resides in the intestine. These two forms are affected differently by inhibitors. Levamisole added to the substrate inhibits endogenous non-intestinal alkaline phosphatases but does not affect the intestinal form commonly used as a detection label.

**Note:** Levamisole-resistant alkaline phosphatase has been detected in four different types of tumor cells.

## Procedure for Immunohistochemical Staining

1. Block nonspecific sites in the tissues with normal serum or other blocking buffer such as Thermo Scientific SuperBlock Blocking Buffer in TBS (Product No. 37535) for 30 minutes at room temperature in a humidity chamber. Drain the blocking buffer but do not remove excess.
2. Incubate tissues with primary antibody for 30 minutes at room temperature in a humidity chamber.
3. Wash tissues in Tris-buffered saline (TBS) for 3 minutes. Repeat wash step. Remove excess wash buffer.
4. Incubate tissues with alkaline phosphatase conjugated secondary antibody for 30 minutes at room temperature in a humidity chamber.
5. Wash tissues with TBS for 3 minutes. Repeat wash step. Remove excess wash buffer.
6. Mix the 1-Step NBT/BCIP plus Suppressor and add it to the tissue until desired stain develops.
7. Rinse slides with water.

## General References

- Van Belle, H. (1972). Kinetics and inhibition of alkaline phosphatases from canine tissues. *Biochim Biophys Acta* **289**:158-68.
- Van Belle, H. (1976). Kinetics and inhibition of rat and avian alkaline phosphatases. *Gen Pharmac* **7**:53-8.
- Van Belle, H. (1976). Alkaline phosphatases, I, Kinetics and inhibition by levamisole of purified isoenzymes from humans. *Clin Chem* **22**:972-6.
- Ponder, B.A. and Wilkinson, M.M. (1981). Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. *J Histochem Cytochem* **29**:981-4.
- Morris, R.E. and Horowitz, D.A. (1984). Cellular enzyme-linked immunospecific assay (CELISA) IV. Inhibition of endogenous cellular alkaline phosphatase activity. *J Immun Meth* **68**:11-17.
- Janckila, A.J., et al. (1985). Immunoalkaline phosphatase cytochemistry. Technical considerations of endogenous phosphatase activity. *Am J Clin Pathol* **84**:476-80.

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