



## BCIP and NBT Storage in High-Density Polyethylene Bottles

**Purpose:**

To evaluate the stability of the BCIP Concentrate Solution and the NBT Concentrate Solution in amber Nalgene high-density polyethylene (HDPE) bottles over time.

**Reagents:**

This study involved placing representative samples of the substrate solutions in both HDPE and glass (control) bottles. Samples were placed at 4°C, room temperature, and 37°C for 6 months. The following lots of substrate solutions were used:

<u>Substrate Solution</u>	<u>Lot Number</u>
BCIP Concentrate Solution	LA01
NBT Concentrate Solution	LA02

**Test Parameters:**

The components are evaluated using a dot ELISA test procedure. The assays are performed on standard nitrocellulose (Schleicher & Schuell) as follows:

1. Set up dilution plate by performing 12 two-fold dilutions across a single row of a microtiter plate with Mouse IgG (Cappel), starting at a 0.1 mg/ml concentration in PBS.
2. Using an appropriate pen, mark nitrocellulose by making grid.
3. Wet nitrocellulose with reagent quality water.
4. From each well in the dilution plate, transfer 1.0 µl of the diluted Mouse IgG to appropriate spot on gridded membrane strips using a microdispenser. Incubate strips for approximately 5 minutes to allow protein to adhere to the membrane.
5. Block strips with 1% BSA for 15 minutes at room temperature.
6. Incubate strips with Phosphatase-labeled Goat anti-Mouse IgG (H+L), Catalog No. 15-18-06 (Lot LC44-5) diluted 1:2500 in BSA Diluent /Blocking Solution (Cat. No. 50-61-00) for 1 hour at room temperature.
7. Wash strips 3 times with 3 minutes soak periods using Wash Solution concentrate (Cat. No. 50-63-00). After final wash, rinse strips with water.
8. Prepare BCIP/NBT Substrate by combining 10 parts 0.1M Tris Buffer Solution (Product Code 50-81-01) with 1 part BCIP Concentrate Solution and 1 part NBT Concentrate Solution. All test samples of BCIP Concentrate Solution were mixed with a sample of NBT Concentrate (lot LA02) stored at 4°C. Test samples of NBT Concentrate Solution were mixed with a sample of BCIP (lot LA01) stored at 4°C.
9. Stop substrate reaction after 10 minutes by rinsing nitrocellulose in water for 10-20 seconds.
10. Allow strips to air dry before storing under clear plastic.

**Results:**

In this study, samples of BCIP Concentrate Solution and NBT Concentrate Solution showed no significant change in sensitivity as a result of storage in the HDPE bottles over a 6 month period. Storage at elevated temperatures (room temperature and 37°C) did not result in significant loss of sensitivity.