



## STABILITY OF ONE COMPONENT BCIP/NBT SUBSTRATE

**Purpose:**

To evaluate the stability of One Component BCIP/NBT Phosphatase Substrate stored at 4°C and room temperature over time.

**Reagents:**

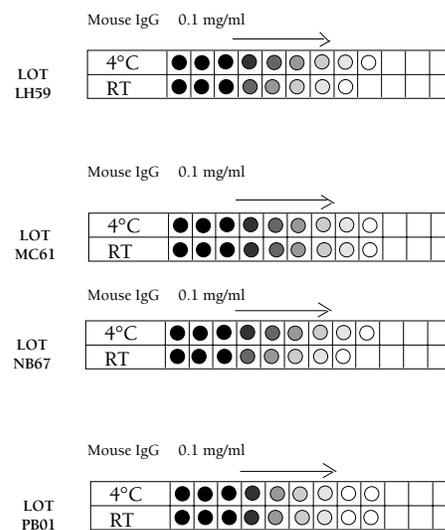
This study compares the performance of four lots of One Component BCIP/NBT substrate solution. Representative samples from each of the following lots were stored at 4°C and room temperature from the date of manufacture..

<u>Lot Number</u>	<u>Date of Mfg.</u>
LH59	8/89
MC61	3/90
NB67	2/91
PB01	2/92

**Test Parameters:** The substrates were evaluated using a dot ELISA test procedure. The assays were performed on standard nitrocellulose membrane (Schleicher and Scheull) as follows:

1. Prepare two-fold dilutions of Mouse IgG (Cappel, Lot 34819) in a microwell ELISA plate, starting at a 0.1mg/ml concentration in PBS.
2. Mark the nitrocellulose membrane with a grid (Figure 1.), using an appropriate pen.
3. Wet the nitrocellulose membrane with reagent quality water.
4. Transfer 1.0 µl of the diluted Mouse IgG from each well in the dilution plate to the appropriate spot on the duplicate gridded membrane strips using a microdispenser. Air dry strips for approximately 5 minutes to allow protein to adhere to the membrane.
5. Block strips with 1% BSA Diluent/Blocking Solution (Cat. No. 50-61-00), Lot NK22, for 15 minutes at room temperature.
6. Incubate strips with Phosphatase-labeled Goat anti-Mouse IgG (H+L), (Cat. No. 15-18-06), Lot KE10-5, diluted 1:2500 in BSA Diluent /Blocking Solution, for 1 hour at room temperature.
7. Wash strips with a 15 minute soak period using Wash Solution Concentrate (Cat. No. 50-63-00), Lot MH42, diluted 1:20 in reagent quality water. Rinse strips with reagent quality water after washing.
8. Place strips in the appropriate one component BCIP/NBT substrate solution and incubate 10 minutes at room temperature.
9. Stop substrate reaction after 10 minutes by rinsing the membrane in water for 10-20 seconds.
10. Allow strips to air dry. Store sealed under plastic in the dark.

Figure 1.



**Results:**

In this study, all samples of one component BCIP/NBT Phosphatase Substrate stored at 4°C show no significant variation in sensitivity (Figure 1) or physical appearance. Samples stored at room temperature show a loss in sensitivity of one two-fold dilution of Mouse IgG for lots NB67 and LH59 (Figure 1). All samples stored at room temperature contained a fine black precipitate, and these solutions were brighter yellow in color than the samples stored at 4°C.

**Conclusions:**

KPL's One Component BCIP/NBT Phosphatase Substrate is very stable over time when stored at the recommended 4°C . This product also appears relatively stable when stored at room temperature, showing only a slight loss in activity over time