Technical Service Report

Comparison of BCIP/NBT Phosphatase Substrates: One vs. Three Component Systems

Purpose:

To compare the performance of KPL one and three component BCIP/NBT Phosphatase Substrate Systems.

Reagents:

Substrate Components	Product Code	Lot Number	Date of Manufacture
BCIP/NBT One Component	50-81-08	NH52	8/91
BCIP/NBT One Component	50-81-08	NM41	12/91
BCIP Concentrate	50-81-02	NB66	2/91
BCIP Concentrate	50-81-02	NM09	12/91
NBT Concentrate	50-81-03	NM10	12/91
Tris Buffer	50-81-01	NM08	12/91

Procedure:

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

1. Prepare two-fold dilutions of Human IgG (Cappel Lot 34428) in a microwell ELISA plate, starting at a concentration of 0.1 mg/ml in PBS.
2. Mark the nitrocellulose membrane with a grid (Figure 1), using an appropriate pen.
3. Wet the membrane with reagent quality water.
4. Transfer 1.0 µl of the diluted Human IgG from each well in the dilution plate to the appropriate spot on duplicate gridded membrane strips using a microdispenser. Air dry strips for approximately 5 minutes to allow protein to adhere to the membrane.
5. Dilute BSA Diluent/Blocking Solution Concentrate (Product Code 50-61-01), Lot NK22, 1:10 in reagent quality water.
6. Block the strips in 1% BSA Diluent/Blocking Solution for 15 minutes at room temperature.
7. Incubate strips with Phosphatase-Labeled Goat Anti-Human IgG (H+L), (Catalog No. 15-10-06), Lot NL50-5, diluted 1:2500 (0.2 µg/ml) in 1% BSA Diluent/Blocking Solution, for 1 hour at room temperature.
8. Wash strips with a 15 minute soak period using Wash Solution Concentrate (Product Code 50-63-02), Lot NH27. Rinse strips with reagent quality water after washing.
9. Prepare three component BCIP/NBT substrate working solutions by adding one part BCIP Concentrate (Lot NB66 or NM09) and one part NBT Concentrate (Lot NM10) to ten parts Tris Buffer (Lot NM08). Prepare separately for each lot of BCIP Concentrate.
10. Incubate duplicate strips in the appropriate substrate solution for 5 minutes.
11. Stop substrate reaction after 5 minutes by rinsing the strips in water for 10-20 seconds.
12. Allow strips to air dry. Store sealed under plastic in the dark.

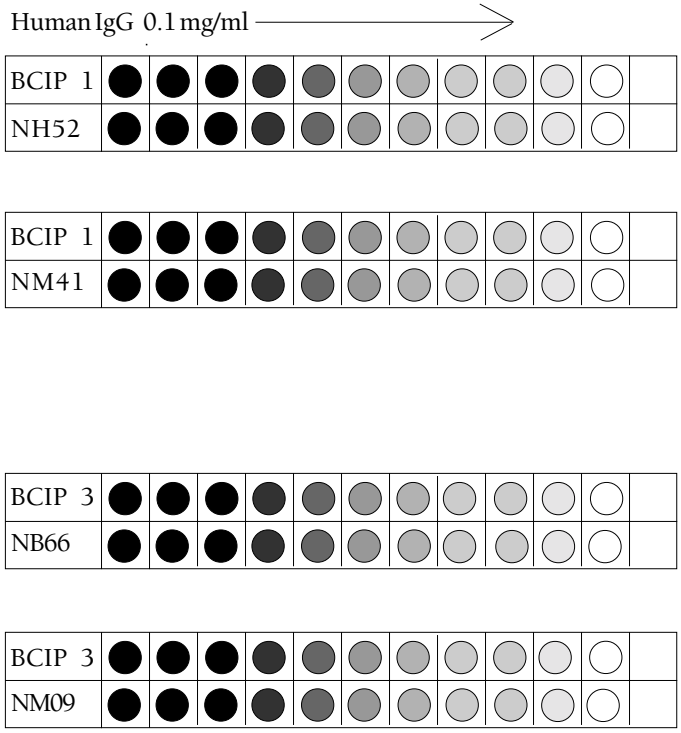
Results:

Human IgG was detected to the same endpoint concentration (0.1 µg/ml) by all lots of BCIP/NBT substrate (See Figure 1). Equally intense color development was observed for both the one and three component systems. All strips exhibited minimal background staining.

Conclusions:

KPL's One and Three Component BCIP/NBT Phosphatase Substrate Systems are equally sensitive and consistent in performance.

Figure 1.



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