



Stability of the BCIP/NBT Substrate System

Purpose:

To compare the sensitivity of various lots, produced over a three year period, of BCIP solutions and NBT solutions and any differences between 4°C and room temperature storage conditions.

Reagents:

BCIP Solutions (4°C and RT)

<u>Lot No:</u>	<u>Date of Mfg.</u>
KH37	9/88
JB30	2/87
HA47	1/86
GC30	3/85

NBT Solutions (4°C and RT)

<u>Lot No:</u>	<u>Date of Mfg.</u>
KH38	9/88
JB29	2/87
HA48	1/86
GC31	3/85

Observations: Reagents start out colorless and a blue precipitate forms as a function of time and elevated temperatures.

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Test Parameters:

Stability study used a dot ELISA procedure. Human IgG (Lot 29495, Cappel) starting at 1mg/ml was titrated through 12 two-fold dilutions on a ELISA plate. 1µl of each dilution was then applied to 14 nitrocellulose strips. This resulted in a range of antigen applied to the strips of 1000 ng for the first dilution to 0.5 ng for the final dilution. Strips were blocked in 1% BSA Diluent/Block Solution for 15 minutes. Strips were then incubated for 1 hour in Phosphatase-Labeled Goat Anti-Human IgG (g) (lot KD24-1) at a concentration of 0.2 µg/ml. Strips were then washed for 15 minutes with Wash Solution Concentrate. Each nitrocellulose strip received a different combination of substrate lots:

<u>Strip</u>	<u>BCIP</u>	<u>NBT</u>
1A and 1B	KH37	KH38
2A and 2B	JB30	KH38
3A and 3B	HA47	KH38
4A and 4B	GC30	KH38
5A and 5B	KH37	JB29
6A and 6B	KH37	HA48
7A and 7B	KH37	GC31

- Strips 1A-7A used BCIP and NBT reagents stored at room temperature.

- Strips 1B-7B used the same lots of BCIP and NBT stored at 4°C.

0.1M Tris Buffer Solution (lot KC64) was used for all strips. All strips were stopped with water after a 4.5 minute substrate incubation period.

Results:

The sensitivity of the various combinations of BCIP/NBT solutions proved to be very consistent. Strip 1A treated with lot KH37 (BCIP) and KH38 (NBT) seemed slightly more sensitive, detecting Human IgG antigen at levels of approximately 0.5 ng. The other strips detected antigen levels between 0.5-2.0 ng. There was no significant difference between the reagents stored at room temperature (strips 1A-7A) and the corresponding reagents stored at 4°C (strips 1B-7B).

Conclusions:

KPL's BCIP/NBT substrate system proved to be highly stable. There was no significant change in sensitivity between lots manufactured over a three year period. Furthermore the BCIP/NBT substrate system proved equally stable at both room temperature and 4°C. The presence of the blue precipitate in the BCIP Solution did not affect product performance.

ML-115-02