Technical Service Report

Stability of Phosphatase Substrate *p*NPP Tablets

Purpose:

To evaluate the performance of *p*NPP (*p*-nitrophenylphosphate) tablets over time when stored at 4°C .

Reagents:

This study compares the performance of six lots of *p*NPP Tablets. Representative samples of each of the following lots were stored at 4°C from the date of manufacture.

Lot Number	Date of Mfg.
GF02	5/85
HL33	11/86
JK15	10/87
KG38	7/88
LJ03	9/89
MH63	8/90

Test Parameters:

The substrates were evaluated using a microwell ELISA test procedure as follows:

1. Add 100 µl Human IgG (Cappel; Lot 34428) diluted to 10 ug/ml in PBS to all test wells in Rows A-G (See Fig. 1). To each test well in Row H add 100 µl of PBS. Incubate one hour at room temperature.
2. Block the plate by adding 300 µl of BSA Diluent/Blocking Solution Concentrate (diluted 1:10 in reagent quality water) to all test wells. Incubate for five minutes at room temperature.
3. Prepare a 1:500 dilution of Phosphatase-labeled Goat anti-Human IgG (H+L), Lot NB50-5, by adding 20 µl of conjugate to 10 ml of 1X BSA Diluent/Blocking Solution.
4. Add 200 µl of the diluted conjugate to the appropriate wells in Row A. Add 100 µl of 1X BSA Diluent/Blocking Solution to all test wells in rows B-H. Perform serial two-fold dilutions of the conjugate by transferring 100 µl from each test well in Row A to the well below. Continue to make dilutions through Row H.
5. Incubate for one hour at room temperature.
6. Wash plate 5 times with Wash Solution Concentrate (Lot NA90) using an automatic Skatron plate washer.
7. In separate test tubes, prepare each phosphatase substrate sample by mixing 3 ml DEA Phosphatase Substrate Solution (Lot MH58) with 12 ml reagent quality water, and then adding 3 *p*NPP tablets from each test lot.
8. Add 100 µl of substrate solution to the appropriate test wells.
9. The O.D. for each well is determined by the Dynatech MR650 ELISA reader at 410 nm.

Results:

All *p*NPP lots tested gave very similar results in the ELISA (see Fig. 1). Lot JK15, which had turned a darker yellow than the other lots of tablets, gave slightly higher O.D. readings for both the positive and BSA control wells. Lot KG38 tablets were the palest of the lots tested and gave the lowest positive and negative O.D. readings. However, all lots are very similar in activity and there is no clear trend of a decline in activity over time.

Conclusions:

KPL's *p*NPP tablets appear to perform consistently over the six year study period. Lots which have become dark in color appear to give higher positve and negative O.D. readings in ELISA than lots which remain pale in color, but the difference is not significant.

Figure 1

				GF02	HL33	JK15	KG38	LJ03	MH63			
	1	2	3	4	5	6	7	8	9	10	11	12
A				1.075	1.106	1.209	1.022	1.151	1.054			
B				0.598	0.612	0.667	0.570	0.628	0.619			
C				0.347	0.359	0.401	0.338	0.374	0.353			
D				0.218	0.225	0.259	0.216	0.233	0.221			
E				0.153	0.159	0.193	0.156	0.163	0.156			
F				0.124	0.132	0.159	0.123	0.125	0.122			
G				0.107	0.112	0.141	0.106	0.107	0.107			
H				0.090	0.095	0.122	0.087	0.090	0.088			

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