Stability of Lyophilized Phosphatase Conjugates

Purpose:

To evaluate the stability of phosphatase conjugates over a six year period. The effects of storing phosphatase conjugate samples in both lyophilized and rehydrated forms were evaluated.

Assay Parameters:

Lyophilized samples from a single lot of phosphatase-labeled Goat anti-Human IgG (H+L) lot GC36-5 were stored at 4°C. A single vial was rehydrated with reagent quality water on 4/2/85 (Day 1) and stored at -20°C. At yearly intervals, the remaining vials were rehydrated and stored at -20°C (See Figure 1 for date of rehydration). Sample performance was evaluated by comparative ELISA and by enzyme kinetics. The components were evaluated using a microwell ELISA test procedure as follows:

Procedures:

1. Add 100 µl Human IgG (Cappel, Lot 34428) at 1 µg/ml in PBS to all wells in rows A-G. Add 100 µl PBS to all wells in row H.
2. Incubate plate one hour at room temperature.
3. Block the plate for five minutes at room temperature with 300 µl BSA Diluent/Blocking Solution Concentrate (10X) Lot NK22, diluted 1/10 in reagent quality water.
4. Add 100 µl of each conjugate sample (diluted to 0.5 µg/ml in BSA solution) to the appropriate wells in rows A-H.
5. Incubate one hour at room temperature.
6. Wash plate 5X with Wash Solution Concentrate (Lot NH20), using an automatic Skatron washer .
7. Add 100 µl/well of *p*NPP Phosphatase Substrate System (One part DEA Buffer 5X, Lot MH58, in four parts reagent quality water, and one *p*NPP tablet, Lot NH18, per 5 ml of solution).
8. After 20 minutes incubation at room temperature, determine the O.D. for each well using the Dynatech MR650 ELISA reader with a 410 nm filter.

Enzyme kinetics were performed as follows:

1. Prepare substrate solution as described for ELISA and place 3 ml of substrate in a clean cuvette.
2. Dilute the phosphatase conjugate sample to 10 µg/ml in PBS.
3. Add 10 µl of the conjugate sample to the substrate in the cuvette.
4. Run the sample on the Perkin-Elmer spectrophotometer at 410 nm. Measure the rate of color development (D O.D.) by determining the slope of the absorbance change over time.
5. Repeat steps 1-4 for each conjugate sample.

Results:

The data shown in Figure 1 illustrates acceptable performance for all samples in ELISA at the end of the six year study period. Overall, samples rehydrated earlier in the study gave a slightly lower positive O.D. response than the more recently rehydrated samples. Extended storage does not appear to elevate the O.D. values of the negative controls, which averaged 0.070 or lower for all samples (Row H).

Enzyme kinetic activity was consistent over the six year period and remained well above the minimum product specification of 1.5 O.D. units/ minute.

Conclusions:

KPL's lyophilized phosphatase conjugates remain extremely stable when stored at 4°C, with no significant loss of activity over a six year period. Optimal product performance is maintained when product is stored in lyophilized form. When rehydrated and stored at -20°C for several years, the product may show a minimal loss in activity, but still performs well above product specifications and is acceptable for use.

ELISA Data

FIGURE 1.

This data compares the ELISA performance of phosphatase conjugate samples rehydrated at intervals over a six year period. The assay was performed on 12/17/91.

	Date of Rehydration											
	4/2/85		4/23/86		4/23/87		4/14/89		5/11/90		12/13/91	
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.130	1.178	1.035	1.001	1.054	1.107	1.105	1.109	1.200	1.234	1.349	1.066
B	1.038	1.021	0.827	0.867	0.931	0.861	1.053	1.038	1.003	1.075	1.109	1.192
C	1.009	0.961	0.874	0.909	0.826	0.935	1.005	1.178	0.958	1.163	1.185	1.163
D	1.060	0.884	0.774	0.853	0.895	0.955	0.974	1.183	1.121	1.130	1.205	1.125
E	1.008	0.869	0.818	0.799	0.892	0.852	1.056	1.086	1.049	1.210	1.145	1.075
F	0.969	0.902	0.729	0.792	0.848	0.912	1.038	1.098	1.038	1.093	1.125	1.265
G	1.054	1.098	0.935	0.833	0.866	0.954	0.985	1.045	1.166	1.183	1.210	1.096
H	0.068	0.068	0.070	0.070	0.073	0.069	0.066	0.066	0.067	0.067	0.064	0.066
AVG OD-Row H	0.945		0.790		0.850		1.002		1.049		1.100	

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