Stability of Alkaline Phosphatase-Labeled Liquid Conjugates

Purpose:

To evaluate the stability of KPL alkaline phosphatase-labeled (AP) liquid conjugates.

Reagents:

Representative samples of the following lots of AP Goat Anti-Mouse IgG (H+L), at 1 mg/ml, were stored at 4°C from the date of manufacture.

Lot Number	Date of Mfg.
PJ54	9/92
QC38	3/93
RF02	6/94
LJ56 lyoph. reference	11/89

Test Parameters:

The conjugates were evaluated using a microwell ELISA procedure. The assay was performed as follows:

1. Add 100 µl Mouse IgG (Cappel; Lot 38621) diluted to 10 µg/ml in PBS to all test wells in columns 1-12. Incubate one hour at room temperature.
2. Block the plate with 300 µl/well BSA Diluent/Blocking Solution (Lot QJ02) diluted 1:10 in reagent quality water. Incubate 10 minutes.
3. Dilute each AP Goat anti-Mouse IgG (H+L) sample to 0.25 µg/ml in 1X BSA Diluent.
4. Add 200 µl of each diluted conjugate sample to the appropriate wells in Row A (see Figure 1). 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 100µl from each 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 RK37) using an automatic Skatron plate washer.
7. Prepare DEA substrate by mixing 3 ml DEA Buffer (Lot PK12) with 12 ml RO water, and then adding 3 *p*NPP tablets (Lot QK01). Add 100 µl/well and incubate 10 minutes at room temperature.
8. Stop reaction by adding 100 µl of AP Stop Solution (Lot PJ32) to each test well.
9. Determine the O.D. for each well using the Bio-Tek EL311 ELISA reader at 405 nm.

Conjugates were also evaluated for specific activity by spectrophotometer as follows:

1. Prepare DEA Substrate as described in Step 7 above.
2. Prepare duplicate dilutions of each conjugate sample at 0.375 µg/ml after multiplying initial concentration by 1500.
3. Place 2.9 ml substrate into cuvette.
4. Add 100 µl diluted conjugate sample to the cuvette. Mix and run spectrum at following parameters:
   - set on T-drive mode
   - length of run=2 minutes
   - samples run at 25°C
   - Ord. max. = 1.0 O.D.
   - Ord. min. = 0.0 O.D.
   - l = 410 nm
5. Repeat and calculate slope for each sample.

Results and Conclusions:

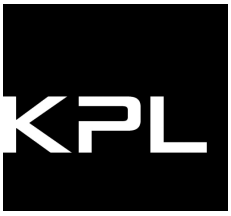
All liquid conjugate samples were equivalent when tested by ELISA (Figure 1), and for specific activity (Figure 2). All samples were higher in activity than the lyophilized reference. This study demonstrates the stability of phosphatase liquid conjugates when stored at 4°C for up to 21 months.

FIGURE 1

	Lot PJ54 4°C		Lot QC38 4°C		Lot RF02 4°C		Lot LJ56 lyoph. ref.					
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.010	2.018	1.885	1.878	2.508	2.538	1.507	1.345				
B	1.191	1.094	1.048	1.043	1.447	1.389	0.799	0.783				
C	0.654	0.612	0.583	0.603	0.858	0.821	0.471	0.472				
D	0.370	0.367	0.353	0.355	0.488	0.480	0.310	0.299				
E	0.249	0.240	0.243	0.239	0.302	0.301	0.220	0.210				
F	0.180	0.179	0.180	0.174	0.210	0.213	0.176	0.160				
G	0.146	0.148	0.147	0.144	0.164	0.164	0.149	0.137				
H	0.131	0.130	0.134	0.128	0.138	0.139	0.134	0.126				

FIGURE 2

Lot Number	Specific Activity
Lot PJ54	24.75
Lot QC38	24.20
Lot RF02	25.70
Lot LJ56	18.10



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