Stability of Peroxidase-Labeled Liquid Conjugates

Purpose:

To evaluate the performance of KPL peroxidase-labeled (HRP) liquid conjugates.

Reagents:

Representative samples of the following lots of liquid HRP 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.
PM24	12/92
QD14	4/93
QE25	5/93
RE23	4/94
RL01	11/94
RB25 lyoph. reference	4/94

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 HRP Goat anti-Mouse IgG (H+L) sample to 0.125 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 100 µl 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 ABTS substrate by mixing equal volumes of ABTS Substrate Solution (Lot QM29) and Solution B (Lot QJ40). Add 100 µl/well and incubate 10 minutes at room temperature.
8. Stop reaction by adding 100 µl of ABTS 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 ABTS Substrate as described in Step 7 above.
2. Prepare duplicate dilutions of each conjugate sample at 0.05 µg/ml.
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.
   - λ = 414 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 lots of conjugate were equivalent in performance. This study demonstrates the stability of HRP liquid conjugates when stored at 4°C for up to 30 months.

FIGURE 1

	Lot PM24		Lot QD14		Lot QE25		Lot RE23		Lot RL01		Lot RB25	
	4°C		4°C		4°C		4°C		4°C		lyoph. ref.	
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.873	1.884	1.801	1.767	1.833	1.877	1.943	1.957	1.933	1.970	1.984	2.041
B	1.416	1.379	1.225	1.230	1.308	1.227	1.489	1.498	1.517	1.575	1.657	1.615
C	0.881	0.847	0.697	0.717	0.791	0.732	0.975	0.965	0.942	1.042	1.112	1.082
D	0.508	0.490	0.386	0.406	0.435	0.399	0.551	0.564	0.563	0.590	0.665	0.629
E	0.302	0.293	0.246	0.240	0.256	0.221	0.318	0.324	0.330	0.347	0.368	0.360
F	0.178	0.177	0.155	0.146	0.159	0.145	0.191	0.195	0.199	0.206	0.225	0.214
G	0.120	0.120	0.109	0.102	0.109	0.101	0.126	0.131	0.132	0.135	0.142	0.143
H	0.095	0.095	0.086	0.080	0.087	0.082	0.098	0.103	0.101	0.099	0.110	0.111

FIGURE 2

Lot Number	Specific Activity
Lot PM24	1079
Lot QD14	1209
Lot QE25	815
Lot RE23	1069
Lot RL01	1376
Lot RB25 (lyoph.)	897

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