

Stability of Liquid Phosphatase Streptavidin

Purpose:

To evaluate the stability of the liquid alkaline phosphatase labeled streptavidin (APSA) cat. #475-3000.

Samples from an R&D pilot lot of APSA were stored at 4°C, room temperature, and 37°C for 14 months. Sample performance was evaluated in an ELISA comparing the R&D pilot lot to a newly prepared manufacturing pilot lot.

<u>Sample</u>	<u>Date of Mfg.</u>
R&D pilot lot	5/7/96
Manufacturing pilot lot #2	6/24/97

Procedure:

Sample performance was evaluated by microwell ELISA as follows:

1. Dilute Biotin labeled Goat Anti-Rabbit IgG(H+L) (Cat. #16-15-06), lot RK70, to 0.5 µg/ml, 0.25 µg/ml, 0.125 µg/ml, 0.0625 µg/ml, 0.0313 µg/ml, 0.0156 µg/ml, 0.0078 µg/ml, and 0.0039 µg/ml in Coating Solution Concentrate (Cat. #50-84-00), lot TK033, diluted 1:10 in reagent quality water. Add 100 µl diluted Biotin Anti-Rabbit IgG(H+L) to all wells and incubate one hour at room temperature.
2. Block plate with 300 µl/well BSA Diluent/Blocking Solution Concentrate (Cat. #50-61-00), Lot TF022, diluted 1:10 in reagent quality water. Block for 15 minutes at room temperature.
3. Dilute AP Streptavidin samples to 0.1 µg/ml in diluted BSA solution. Add 100 µl of APSA to each well and incubate 30 minutes at room temperature.
4. Wash plate 3 times for 3 minutes each with Wash Solution Concentrate (Cat. #50-63-00), Lot RJ45, diluted 1:20 in reagent quality water.
5. Prepare BluePhos[®] Phosphatase Substrate (Cat. #50-88-00) by mixing equal volumes of Solution A, lot TH037, and Solution B, lot TH038. Add 100 µl/well of BluePhos[®] Substrate and incubate 10 minutes at room temperature.
6. Determine the O.D. for each well using a Biotek Ceres 900 ELISA reader with a 620 nm filter.

Results:

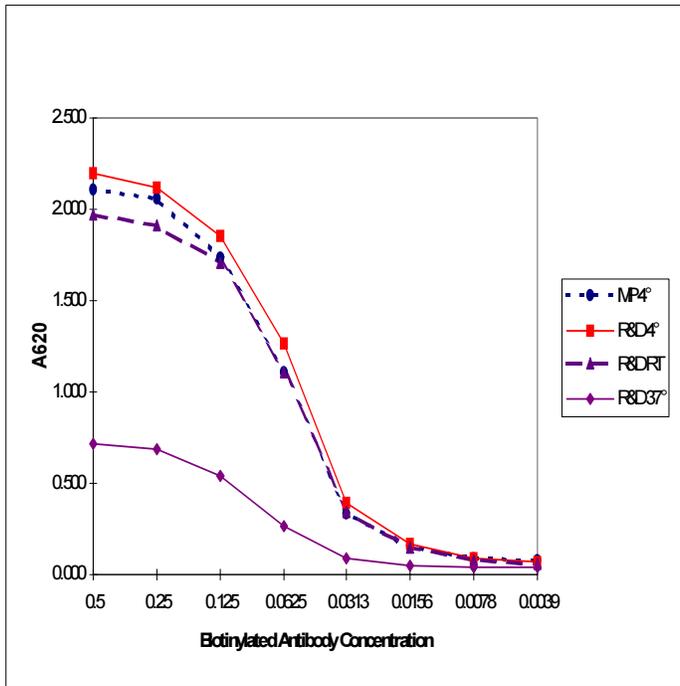
The average value for each sample was calculated (figure 1) and graphed (figure 2). The signal from the manufacturing pilot sample and the R&D 4°C sample look very similar. The R&D room temperature sample gave a slightly lower signal, approximately 10% less, but still performed very well. As expected, the R&D 37°C sample gave a much lower signal, approximately 60% less, but is still active.

Figure 1

B-ab conc.	MP 4°	R&D 4°	R&D RT	R&D 37°
0.5	2.106	2.198	1.969	0.716
0.25	2.059	2.120	1.915	0.684
0.125	1.734	1.856	1.707	0.542
0.0625	1.108	1.266	1.108	0.267
0.0313	0.330	0.397	0.343	0.087
0.0156	0.152	0.166	0.149	0.052
0.0078	0.092	0.090	0.080	0.042
0.0039	0.076	0.065	0.059	0.040

Results: (cont.)

Figure 2



Conclusions:

KPL's liquid phosphatase streptavidin is extremely stable when stored at 4°C, with no significant loss of activity over 14 months. When stored at room temperature, the conjugate shows only a minimal decrease in activity. Storage of the conjugate at 37°C for extended periods of time appears to have an adverse effect on activity. This product is stable for at least 14 months when stored at 4°C.

