# Stability of Lyophilized Peroxidase Conjugates

**Purpose:**

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

**Assay Parameters:**

Lyophilized samples from a single lot of peroxidase-labeled Goat anti-Human IgG (H+L) lot GC06-5 were stored at 4°C. A single vial was rehydrated with reagent quallity water on Year 1 and stored at -20°C. At yearly intervals, the remaining samples 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.

**Procedures:**

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

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 plate 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 µL/mL in BSA solution) to the appropriate wells in rows A-H (Figure 1).
5. Incubate one hour at room temperature.
6. Wash plate 5 times with Wash Solution Concnetrate (Lot NH20), using an automatic Skatron washer.
7. Add 100 µL/well of ABTS Peroxidase Substrate Solution (ABTS Peroxidase Substrate, Lot LL01, mixed with an equal volume of Peroxidase Substrate Solution B, Lot NC60).
8. After 5 minutes incubation at room temperature, determine the O.D. for each well using a Dynatech MR650 ELISA reader with a 410 nm filter.

Enzyme kinetics were performed as follows:

1. Prepare substrate solution as described for ELISA and dispense 3 mL of substrate into a clean cuvette.
2. Dilute the peroxidase conjugate sample to 5 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 417 nm. Measure the rate of color development ($\Delta$ 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 indicates that when tested at the end of the six year study period, all samples performed well in ELISA. All samples had average negative O.D. values of less than 0.070 (Row H). The sample stored in lyophilized form for the entire six year study period gave the highest positive O.D. response. Samples rehydrated in Year 1 and Year 2 showed only slightly lower average positive O.D. values than those of the remaining samples.

Kinetic activity remained well above the minimum product specification of 0.75 O.D. units/minute for all samples. Product which was stored in lyophilized form for the entire study period had enzymatic activity equal to the initial test result on the date of manufacture. A very slight drop in kinetic activity occured in samples which had been rehydrated and stored at -20°C for an extended period of time.

**Conclusions:**

KPL's lyophilized peroxidase conjugates remain extremely stable when stored as recommended at 4°C, with no significant loss of activity over a six year period. The highest level of activity is maintained when product is stored in lyophilized form. When rehydrated and stored at -20°C, the product shows only a minimal decrease in activity over a period of up to six years, and is well within acceptable limits of performance. This study confirms stability of the lyophilized conjugates over the six year study period.

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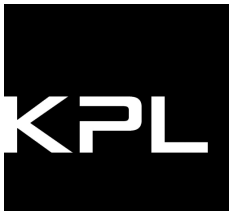
# ELISA Data

| | Year 1 | | Year 2 | | Date of Rehydration Year 3 | | Year 4 | | Year 5 | | Year 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| **A** | 1.118 | 1.146 | 1.146 | 1.057 | 1.129 | 1.292 | 1.295 | 1.110 | 1.104 | 1.139 | 1.389 | 1.311 |
| **B** | 1.478 | 1.021 | 1.097 | 1.102 | 1.118 | 1.159 | 1.224 | 0.181 | 1.135 | 1.286 | 1.338 | 1.405 |
| **C** | 1.308 | 1.129 | 1.072 | 1.133 | 1.195 | 1.148 | 1.356 | 1.324 | 1.335 | 1.208 | 1.348 | 1.352 |
| **D** | 1.190 | 1.052 | 1.086 | 1.104 | 1.210 | 1.254 | 1.195 | 1.324 | 1.232 | 1.126 | 1.277 | 1.328 |
| **E** | 1.314 | 0.989 | 1.137 | 1.155 | 1.243 | 1.213 | 1.286 | 1.335 | 1.059 | 1.129 | 1.171 | 1.409 |
| **F** | 1.114 | 0.958 | 1.043 | 1.110 | 1.181 | 1.186 | 1.171 | 1.146 | 1.054 | 1.206 | 1.142 | 1.299 |
| **G** | 0.951 | 0.964 | 0.914 | 0.955 | 1.009 | 1.099 | 1.135 | 1.157 | 1.059 | 1.229 | 1.188 | 1.120 |
| **H** | 0.062 | 0.065 | 0.063 | 0.061 | 0.063 | 0.065 | 0.068 | 0.061 | 0.060 | 0.060 | 0.056 | 0.058 |
| **AVG OD Row H** | **1.060** | | **1.017** | | **1.110** | | **1.167** | | **1.104** | | **1.234** | |

Figure 1. This figure compares ELISA performance of samples rehydrated at various proints over the six year study period.



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