Technical Service Report

Stabilization of Peroxidase Conjugates in HRP Stabilizer

Purpose:
Evaluate the ability of KPL HRP Stabilizer to maintain enzyme activity of HRP conjugated antibodies over time at various storage temperatures.

Reagents:
Two HRP antibody conjugates were used in this study: HRP Goat anti-chicken IgG (H+L) and HRP anti-p27. HRP Goat anti-chicken IgG (H+L) was diluted to 0.10 mg/mL and HRP anti-p27 was diluted to 0.01 mg/mL for long term storage. Each conjugate was diluted in HRP Stabilizer and 1.0 ml aliquots were taken and stored at 4°C, 25°C, and 37°C. An HRP conjugate diluted in 50% glycerol and stored at –20C was used as a reference.
A commercially available Avian ELISA test kit designed to measure specific antibody levels in sera was used to quantitate the activity of each conjugate.

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Assay Procedure:

1. Add 100 mL of positive control serum diluted 1:100 in Dilution buffer (supplied with kit) to two antigen sensitized microwell plate. Incubate 30 minutes at room temperature.
2. Empty each plate of content and wash 3 times with wash solution (supplied with kit).
3. Retrieve the three, 1ml samples and the reference for both conjugates from their respective storage conditions. Dilute each conjugate 1:100 in Dilution buffer.
4. Add 100 ml of each diluted anti-chicken conjugate to the appropriate wells of plate 1 (recommend 24 replicates for each sample tested). Add 100 ml of each anti-p27 in the same fashion to the appropriate wells of plate 2. Incubate for 30 minutes at room temperature and wash as before.
5. Add 100 ml of ABTS Peroxidase Substrate (supplied with kit) to each well and incubate for 15 minutes.
6. Add 100 ml of ABTS Stop Solution (supplied with kit) to each well.
7. Determine the O.D. for each well was determined on an ELISA plate reader with a 410 nm filter.
8. Calculate the average O.D for each conjugate and express each conjugates activity as % activity relative to the reference conjugate.
   Repeat assay as above at various time intervals to determine stability of Hrp conjugates.

Results:
Figure 1 demonstrates the stability of HRP anti-p27 over a 34 week time period when the conjugate is stored in HRP Stabilizer at 4°C, 25°C, and 37°C. 4°C storage consistently exhibits near 100% enzyme activity relative to the reference conjugate, where as storage at 25°C exhibits, on average a 92% enzyme activity over 34 weeks. Storage at 37°C results in a gradual loss of enzyme activity over time with a total 60% loss of enzyme activity after 34 weeks. Figure 2 demonstrates data for the Hrp anti-chicken conjugate under the same experimental conditions. The results for this conjugate exhibit the same pattern of stability. Storage at 4°C results in maintenance of near 100% enzyme activity over 34 weeks, 25°C results in an average of 95.4% enzyme activity, and 37°C results in a gradual decline in enzyme activity over 34 weeks.

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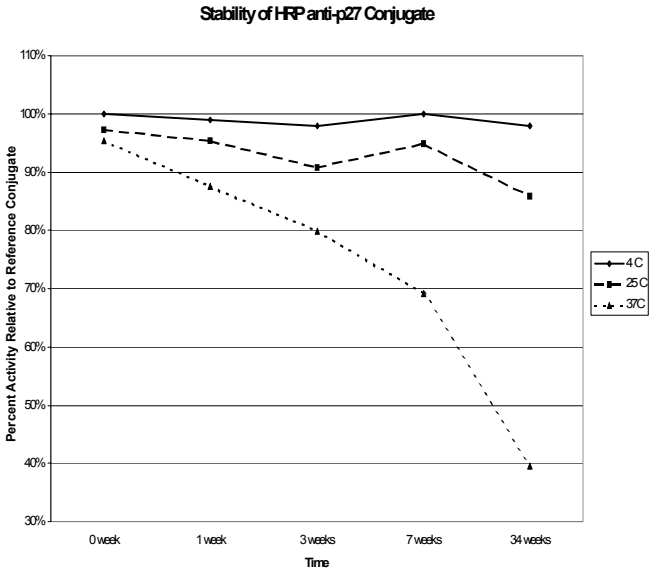

Figure 1: Percent activity of Hrp anti-p27 relative to reference conjugateat 4°C, 25°C, and 37°C over 34 weeks.

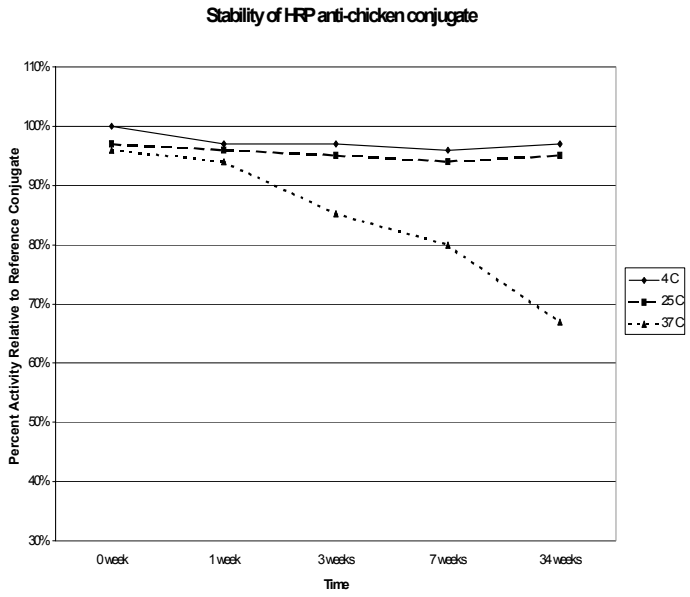

Figure 2: Percent activity of Hrp anti-chicken relative to reference conjugate at 4°C, 25°C, and 37°C over 34 weeks.

Conclusions: The HRP enzyme remains highly stable when stored in HRP stabilizer at either 4°C or 25°C. This confers reliable activity of the enzyme over time when it is stored under these conditions. Hrp conjugates that are stored under these conditions will also provide consistent and reliable results when used in immunological and biological assays.

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