

BEBPA'S 

HALLOWEEN HORRORS



**Assays that have scared
and scarred you!**

Improving HRP Conjugates' stability for use in CMC assays

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Assay Type: Binding Cell-Based Animal-Assay Other

What happened:

CASE 1:

IgG4 Fc, HRP Conjugate: neatly packed in the manufacturer's amber screw-top vial, stored as instructed at 2–8 °C.

Initial titration: OD signal of 1.8 at a 1:24,000

Within just three months, OD of 1.8 dwindled to 0.6; the second facility saw the same decline.

First action: re-titrated HRP, shifting the dilution from 1:24,000 to 1:4,000, and the OD signal back up to 1.83.

Red flag: the two-year stability promised by the manufacturer's COA wasn't holding true in our hands. On top of that, we saw intra-lot variability, with vial readings ranging anywhere from 1.251 to 1.888.

CASE 2:

Another HRP conjugate — a Rat monoclonal Anti-Mouse IgG2a Heavy Chain (HRP) from a second manufacturer — for a different assay, with a reassuring stability claim, suggesting at least one year of acceptable performance under its specifications. Yet within a year, its OD signal had dropped from 2.3 to 1.3



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Additional observations:

The manufacturer's specifications are quite loose: the antibody retains its specificity and remains technically "usable" for ELISA applications

That standard falls short of the requirements for robust, consistent potency assays in CMC.

Solution:

Pooling multiple vials to reduce variability

Storing the conjugates at $-20\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$ in single-use cryovial aliquots

Conclusion:

1. Manufacturers of HRP conjugates apply very broad acceptance criteria for the functional stability of their reagents.
2. Storing the material under conditions that extend its robust performance is the first step towards optimal performance.
3. Freezing temperatures and single-use aliquots, rather than the manufacturer's recommended $2-8\text{ }^{\circ}\text{C}$ and multi-use aliquots, resulted in lots of HRP-conjugated antibodies that perform uniformly and can be used for years.

