

# MossGuard<sup>™</sup> HRP Stabilizing Buffers

Update September, 2023

### Project Goals:

Develop a stabilizing buffer for HRP Conjugates that will allow most HRP conjugates to be supplied in a prediluted form

Greater than 80% reactivity after 60 days storage at 45°C.

Projected 3 year stability of prediluted conjugate at 4ºC.

Equivalent or superior stability compared to industry leaders.

Improved signal and reduced background compared to industry leaders.

Development Program Over 100 different formulations were tested over 9 iterations of optimization and testing

Conjugates were diluted in each formulation at a working concentration.

Solutions were stored at 4°C and 45°C and tested at various timepoints.

After each iteration, modifications were made and new materials were added to be tested in the next iteration.

Stabilzyme (Surmodics) and Guardian (Thermo) were used throughout as control formulations.

Conjugate Source and Characteriz ation

- HRP conjugates of <u>goat-anti human IgG, Fc-</u> <u>specific</u> were purchased from multiple vendors.
- A similar conjugate was made at Moss using the Moss-Link HRP IgG Conjugation Kit.
- The conjugates were titered to generate roughly equal signal when used to detect 1 ng/ml of human lgG.
- Conjugates and concentrations used:
  - Moss: (in-house) (100 ng/ml)
  - Jackson: 109-035-008 (150 ng/ml)
  - Thermo: A18817 (150 ng/ml)
  - Sigma: A0170 (750 ng/ml)
  - Rockland: 609-1303
- (600 ng/ml)

# Standard Assay Protocol

Human IgG was diluted to 1 ng/ml in Assay Buffer.

100 uL of human IgG @ 1/ng/ml was added to a microplate coated with goat anti-human IgG.

The plate was incubated for 60 minutes at room temperature with shaking at 600 rpm.

The plate was washed 3X with 1X PBS-Tween.

100 ul of conjugate solution was added to each well.

The plate was incubated for 30 minutes at room temperature with shaking at 600 rpm.

The plate was washed 5X with 1X PBS-Tween.

100 ul of substrate was added to each well (50% TMBHK)

The plate was incubated for 15 minutes at room temperature.

100 ul of Stop Solution (0.3M H2SO4) was added to each well.

The absorbance at 450 nm was read on a Biotek plate reader.

### Final <u>Formulations</u>

- All versions have equivalent stabilizing properties
- Higher reactivity can reduce conjugate use
- Lower reactivity to match current products

Version	Part No.	Color
MossGuard-M	MGHRP011	Green
MossGuard-T	MGHRP022	Orange
MossGuard-S	MGHRP033	Amber
MossGuard-D	MGHRP044	Red



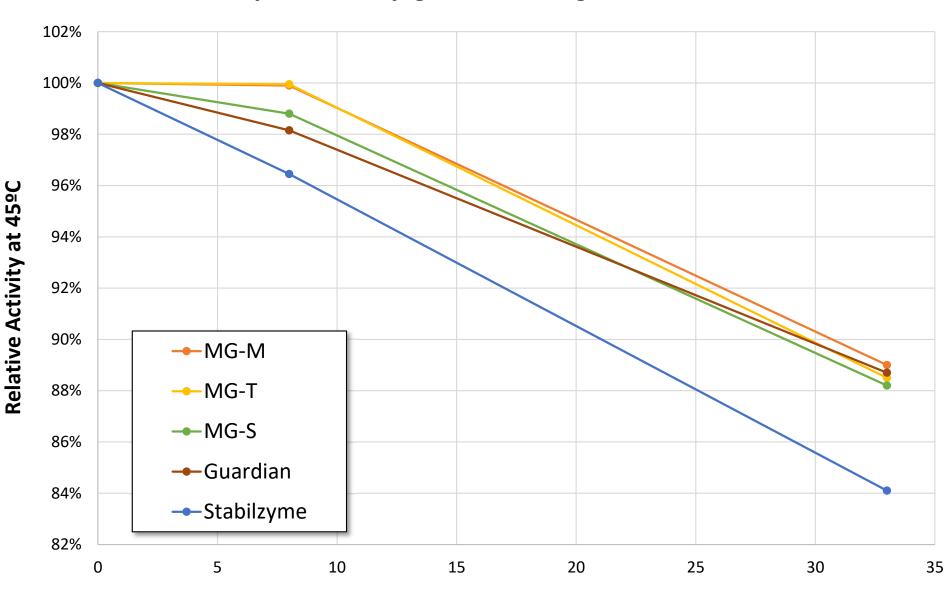






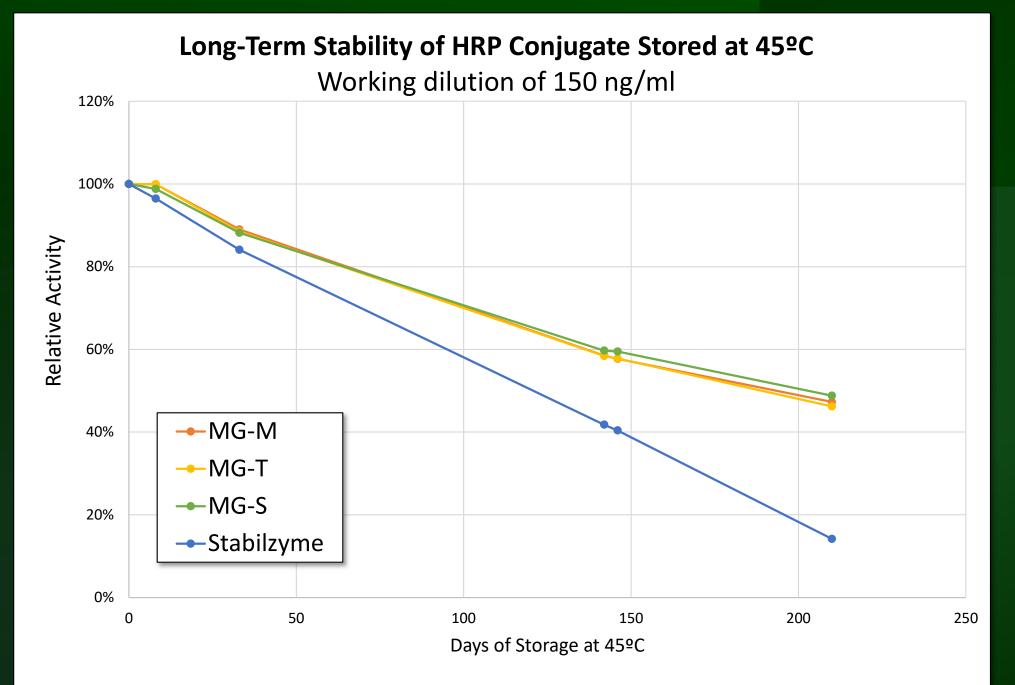






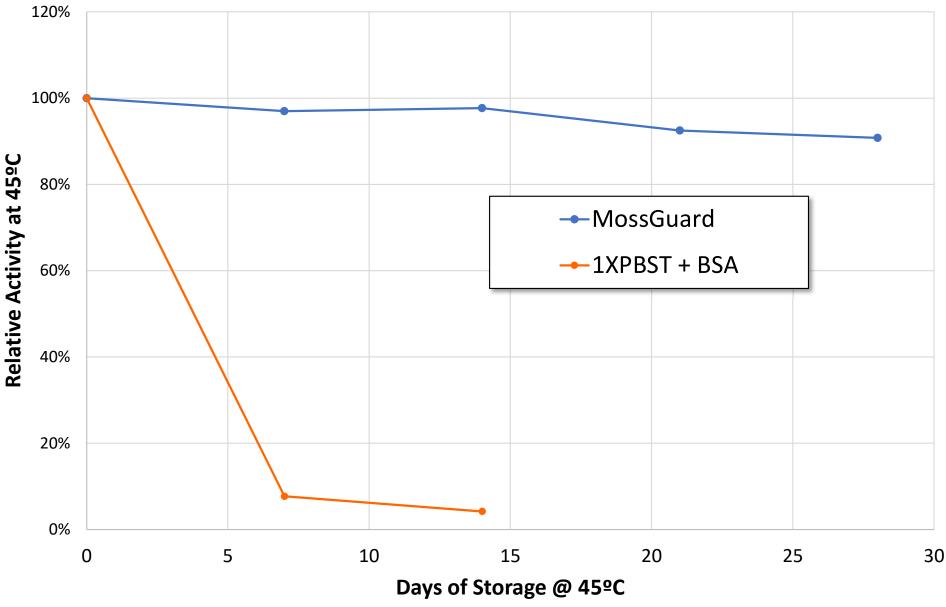
Stability of HRP Conjugate at Working Dilution at 45°C

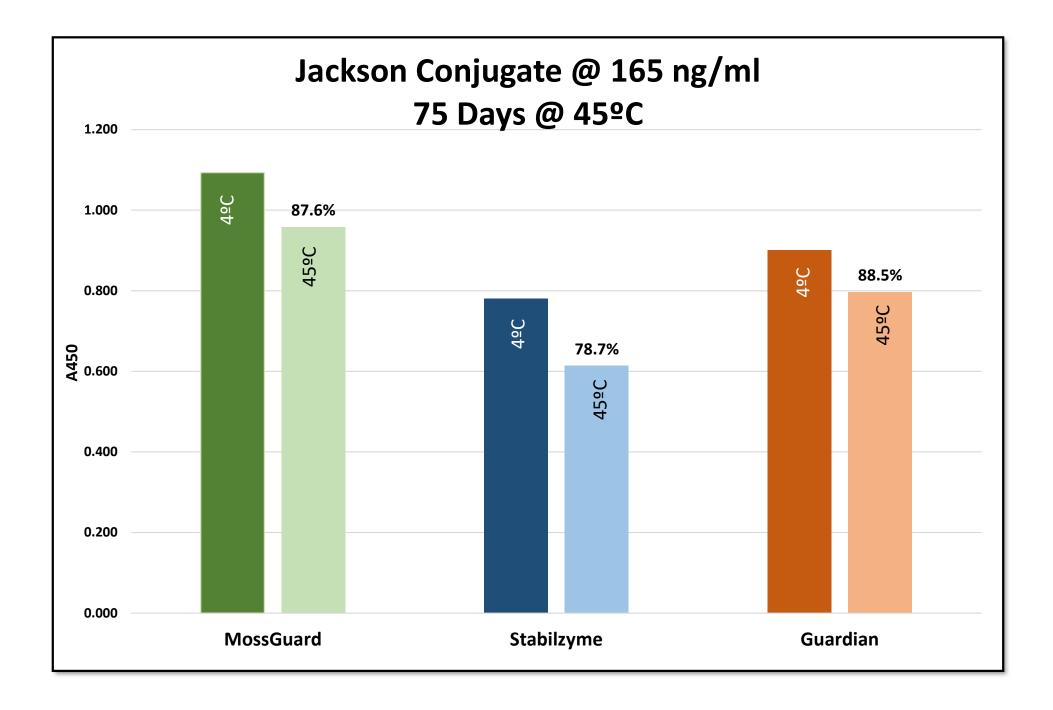
Days of Storage at 45°C

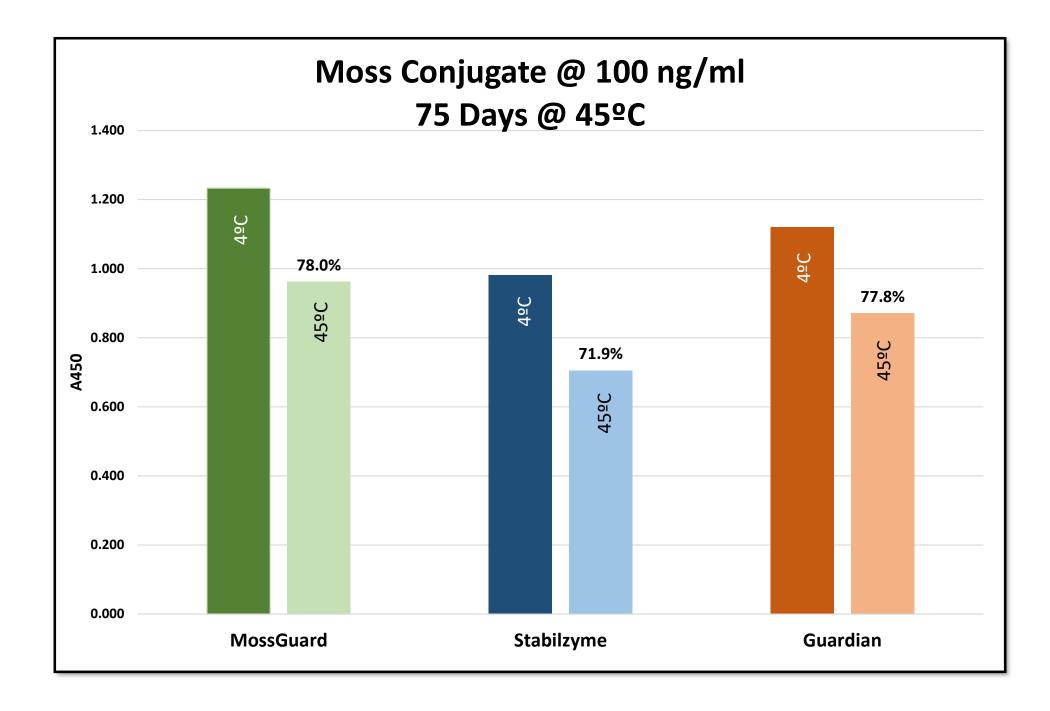


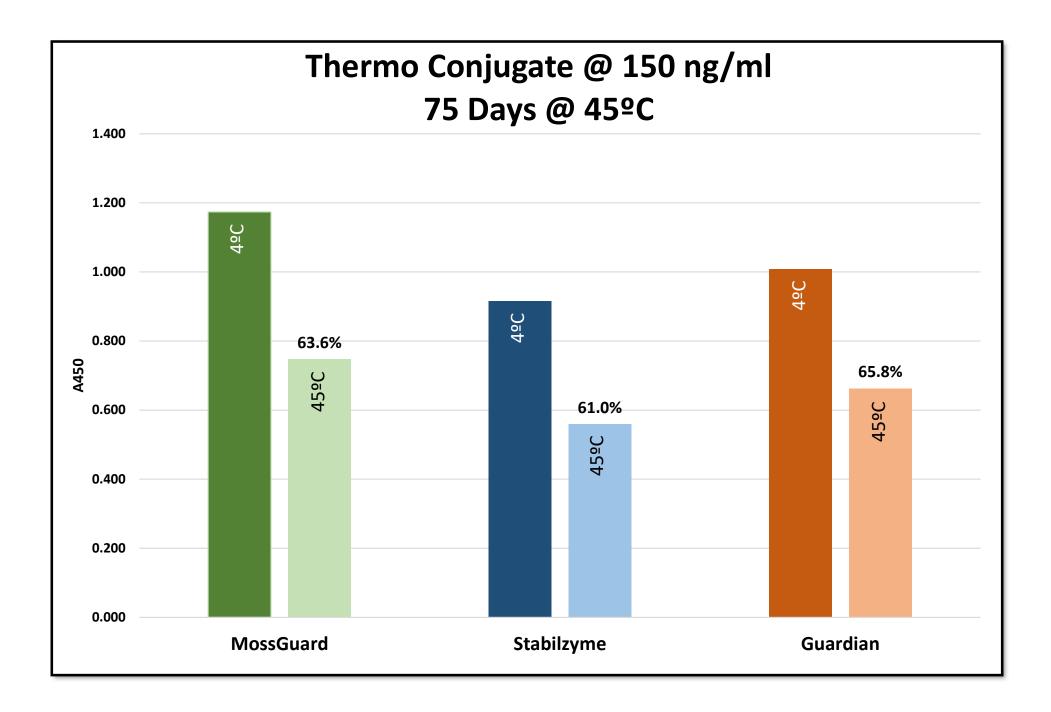
#### Conjugate Stability at 45°C: Comparison of MossGuard to PBST+BSA

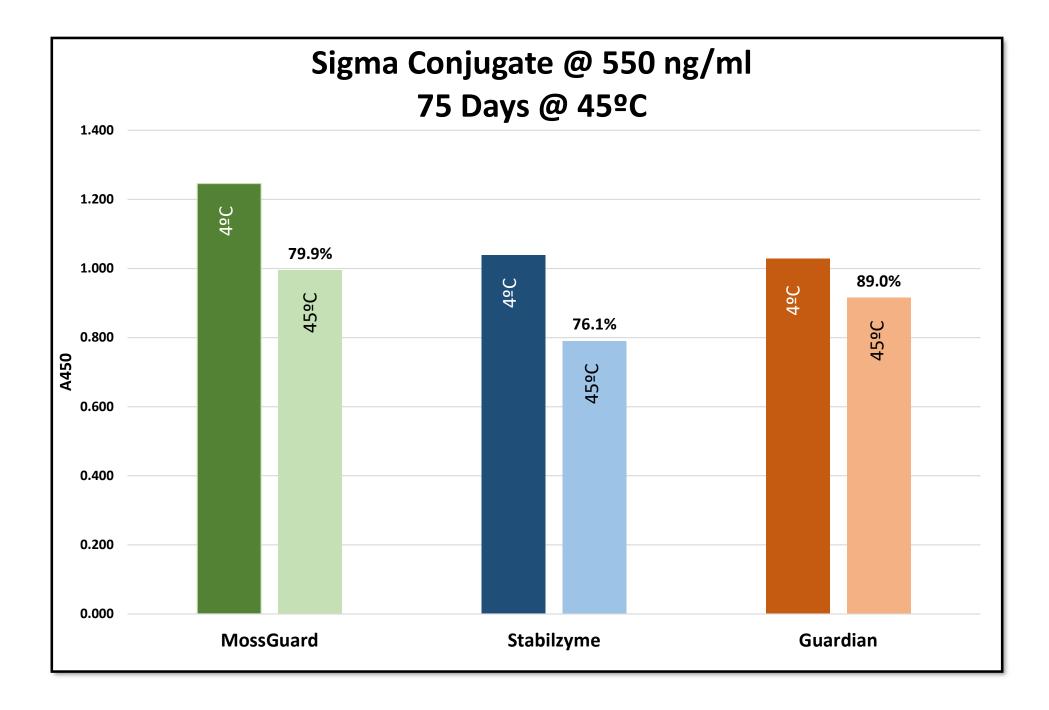
Working conjugate concentration of 150 ng/ml

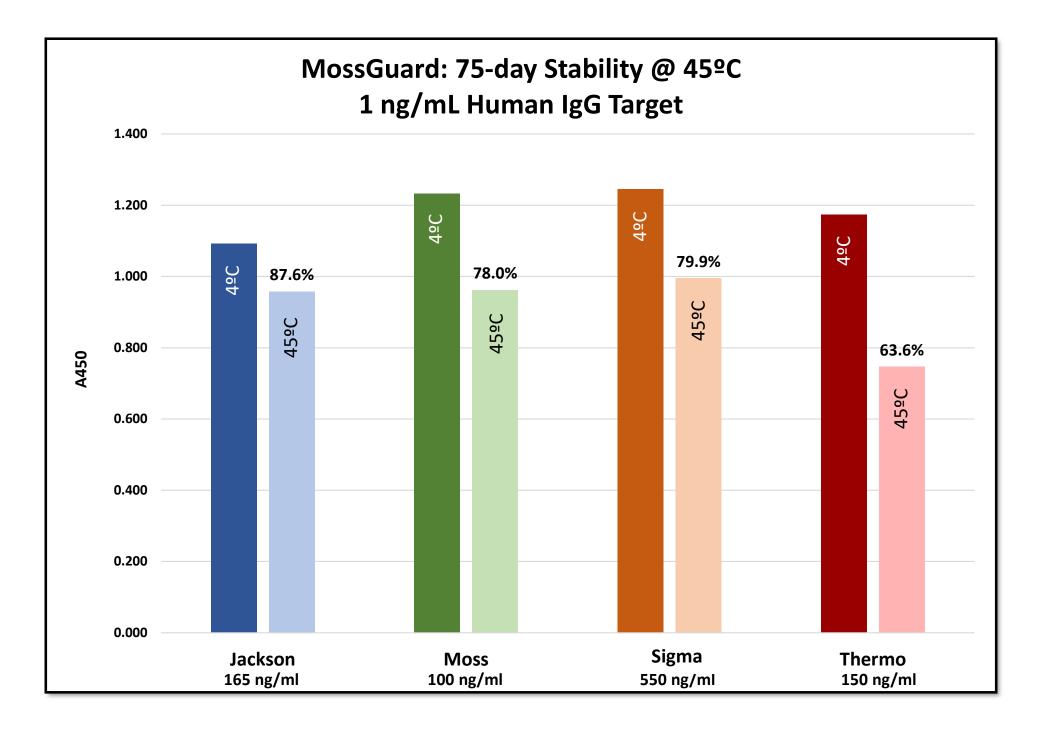


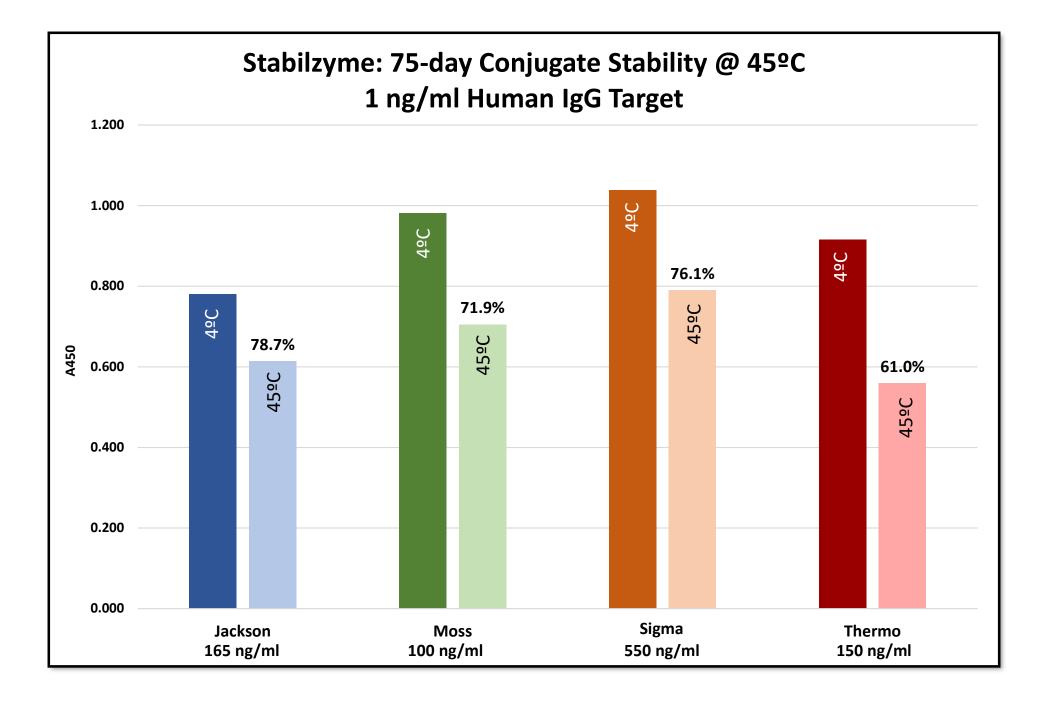


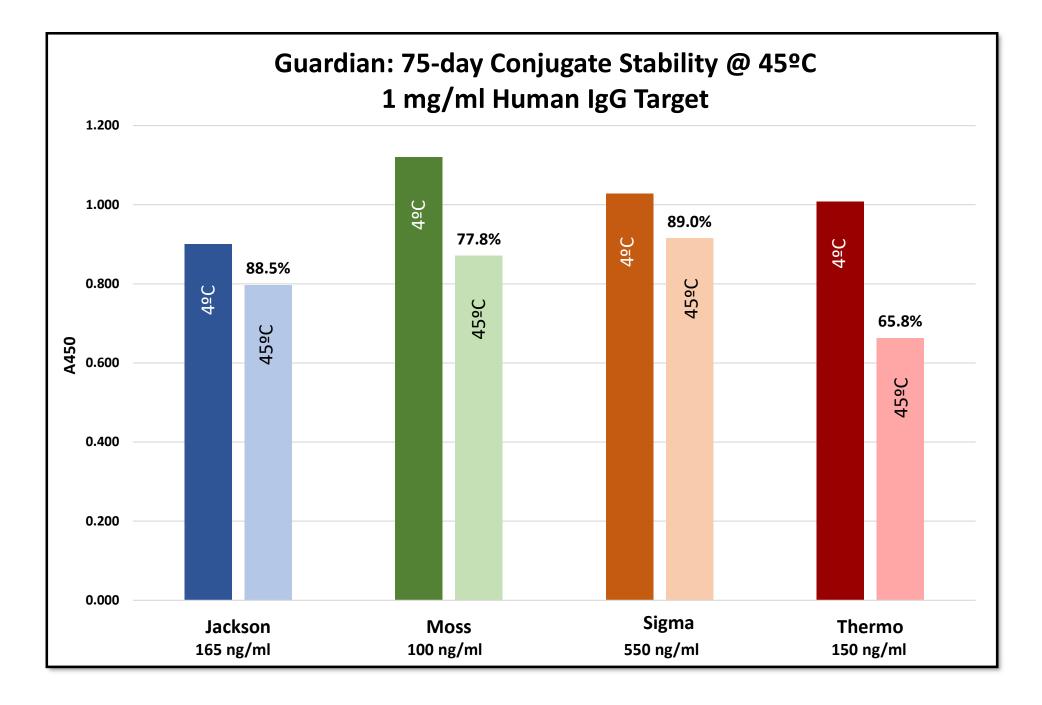












### **Customized Formulation Options for MossGuard Stabilizer**

- Formulation Options
- BSA Concentration can be modified as needed (standard is 5 mg/ml).
- Supplement with animal serum, or additional blocking reagents.
- Alternative preservatives are available (standard is 0.1% ProClin-300).



- Customized Color Options
- Compatible dyes are used to make a range of colors.
- > Uniquely identify your reagent.
- Reduce assay errors with a visible reagent.



### Request a

## MossGuard<sup>™</sup> HRP Test Pack

PN: MGP4A-001

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