Stable Enhanced Chemiluminescent HRP Substrate for ELISA and Western Blot

Jim Lazar and Alvydas Ozinskas.

Moss, Inc. • P.O. Box 189 • Pasadena, MD 21123

www.mossbio.com

Introduction

- Moss, Inc. has developed a new enhanced chemiluminescent substrate (Moss-CHEMI) for HRP.
- Moss-CHEMI is ideal for detection of HRP in ELISA and Western Blot Applications.
- The 2-part substrate has a long shelf life of 18 months when refrigerated.
- A diluent is available to titrate the signal strength to any desired level and to reduce cost.

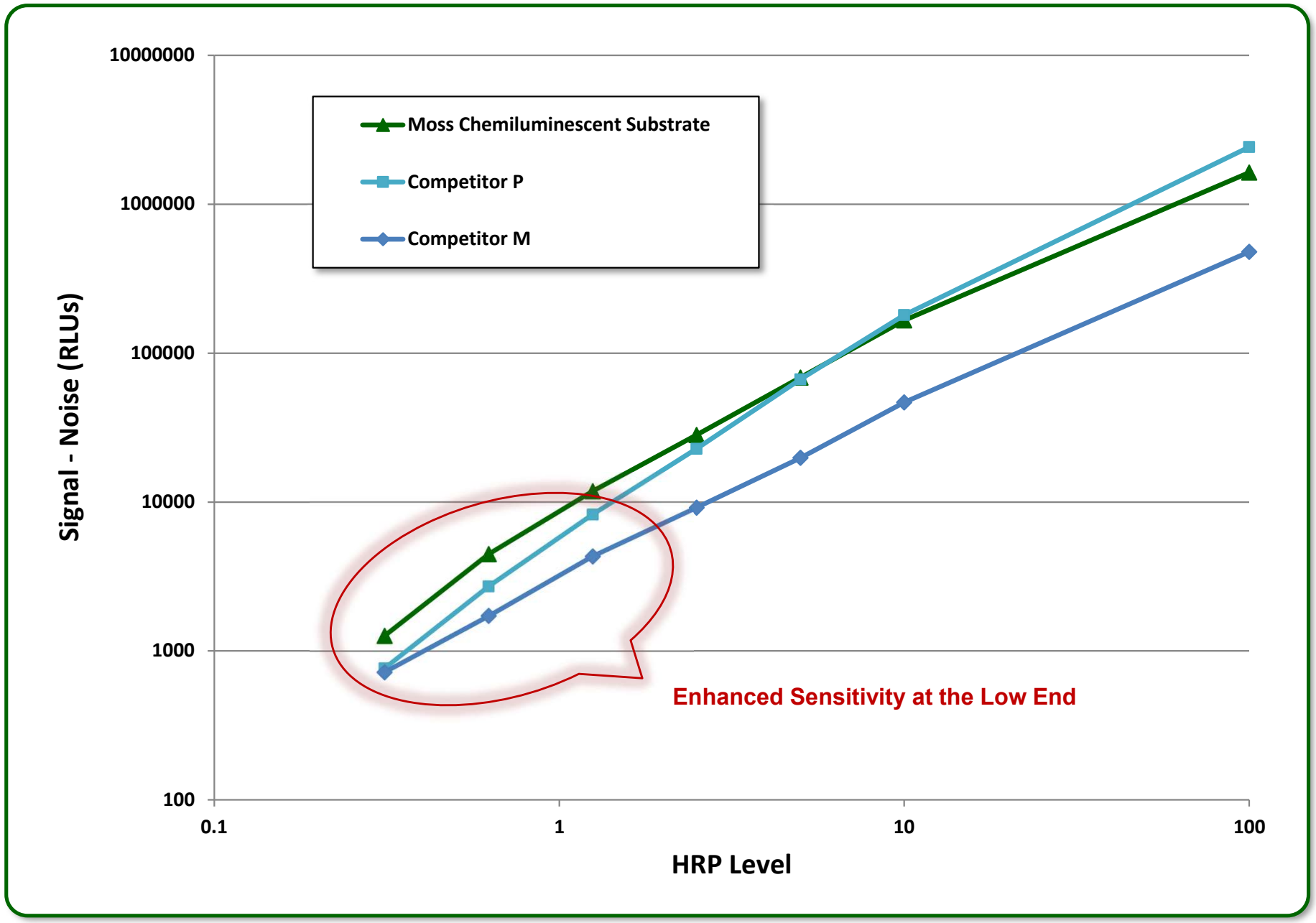
Features and Benefits

- Bright signal produced immediately.
- High sensitivity.
- Reduced assay time.
- Reduced consumption of expensive antibodies and reagents.
- Compatible with nearly all chemiluminescent readers.
- Improved low-end S/N and linearity.
- Stable for 18 months when refrigerated.
- Diluent is available to optimize signal strength and reduce cost.
- Excellent lot-lot reproducibility, produced in large scale, packaged as needed.

Standard Test Protocol

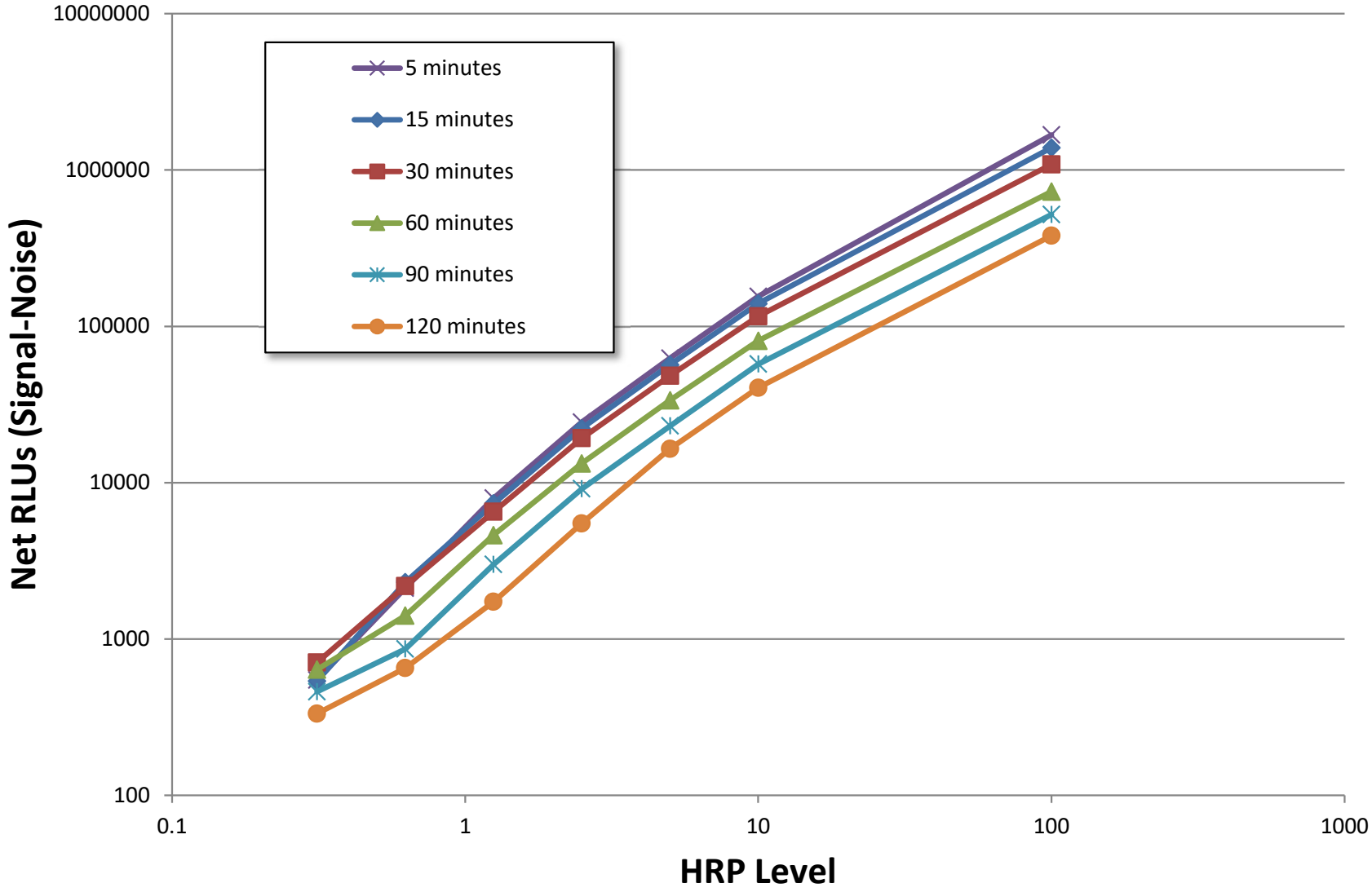
- Peroxidase conjugate is diluted in PBS+0.05% Tween 20.
- Substrates Part A and B are mixed 1:1.
- 100 µL of substrate is added to wells of a black 96-well microplate.
- 5 µL of diluted conjugate is added to the appropriate wells.
- The plate is shaken for 30 seconds at 600 rpm.
- The light output is read on a standard chemiluminescent plate reader (a BioTek FLx800 for this study).

Moss-CHEMI Substrate vs. 2 Leading Competitors



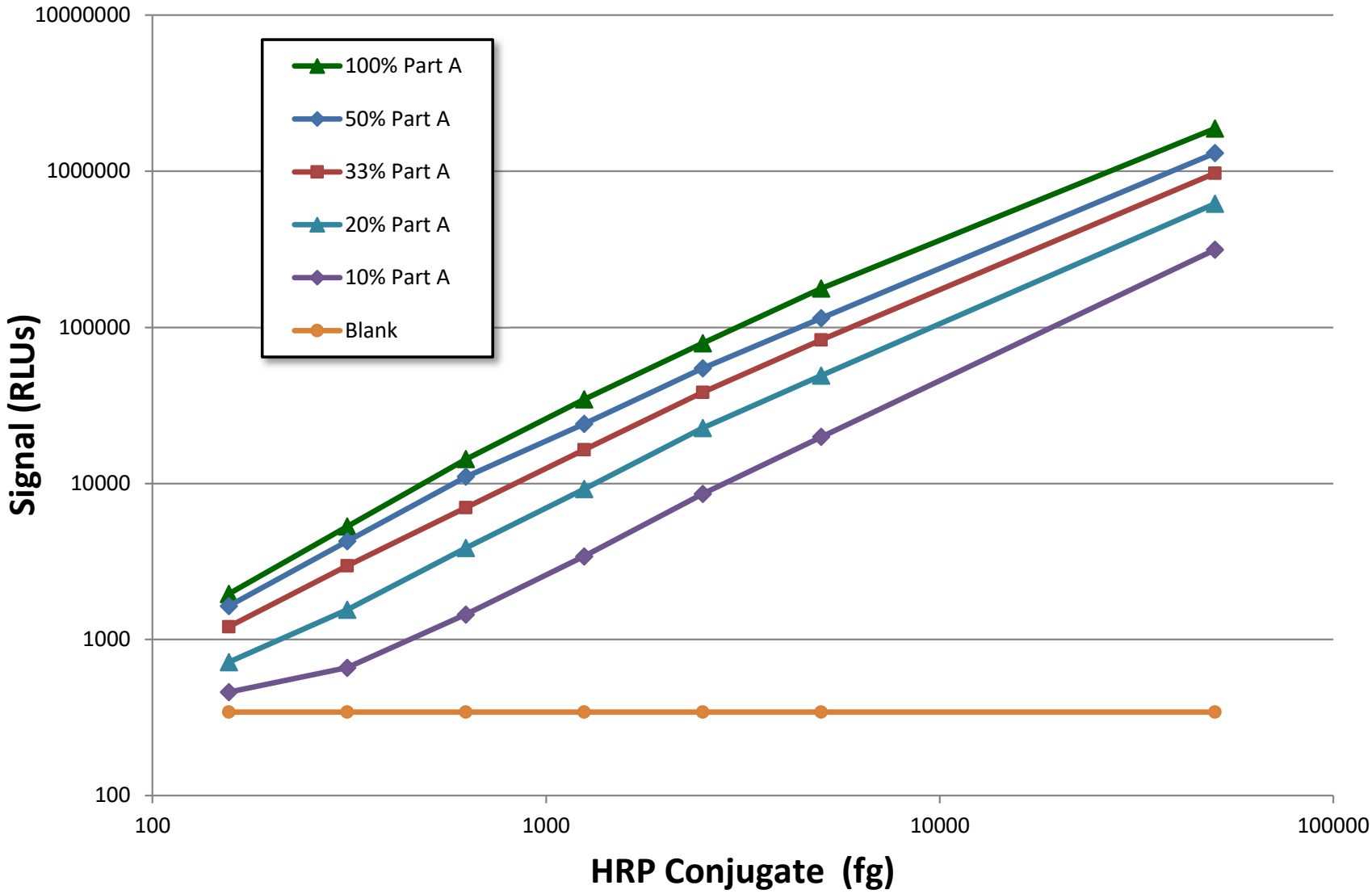
Moss-CHEMI substrate compares favorably to two leading competitors and shows enhanced low-end sensitivity.

Dose Response vs. Substrate Incubation Time



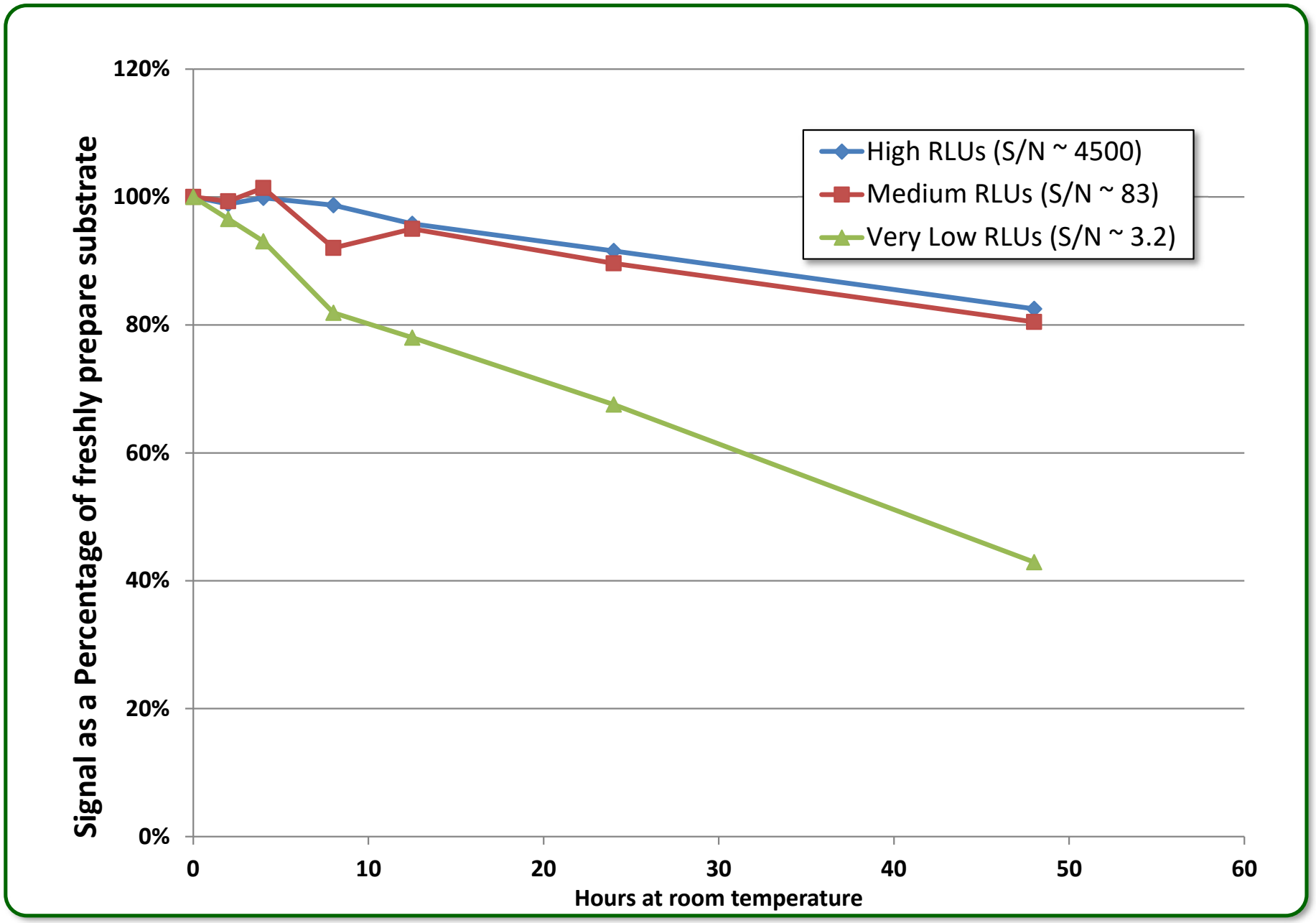
Even after relatively long incubation times (> 30 minutes), the substrate still generates a usable standard curve.

Dilution of Luminol Part A with Diluent

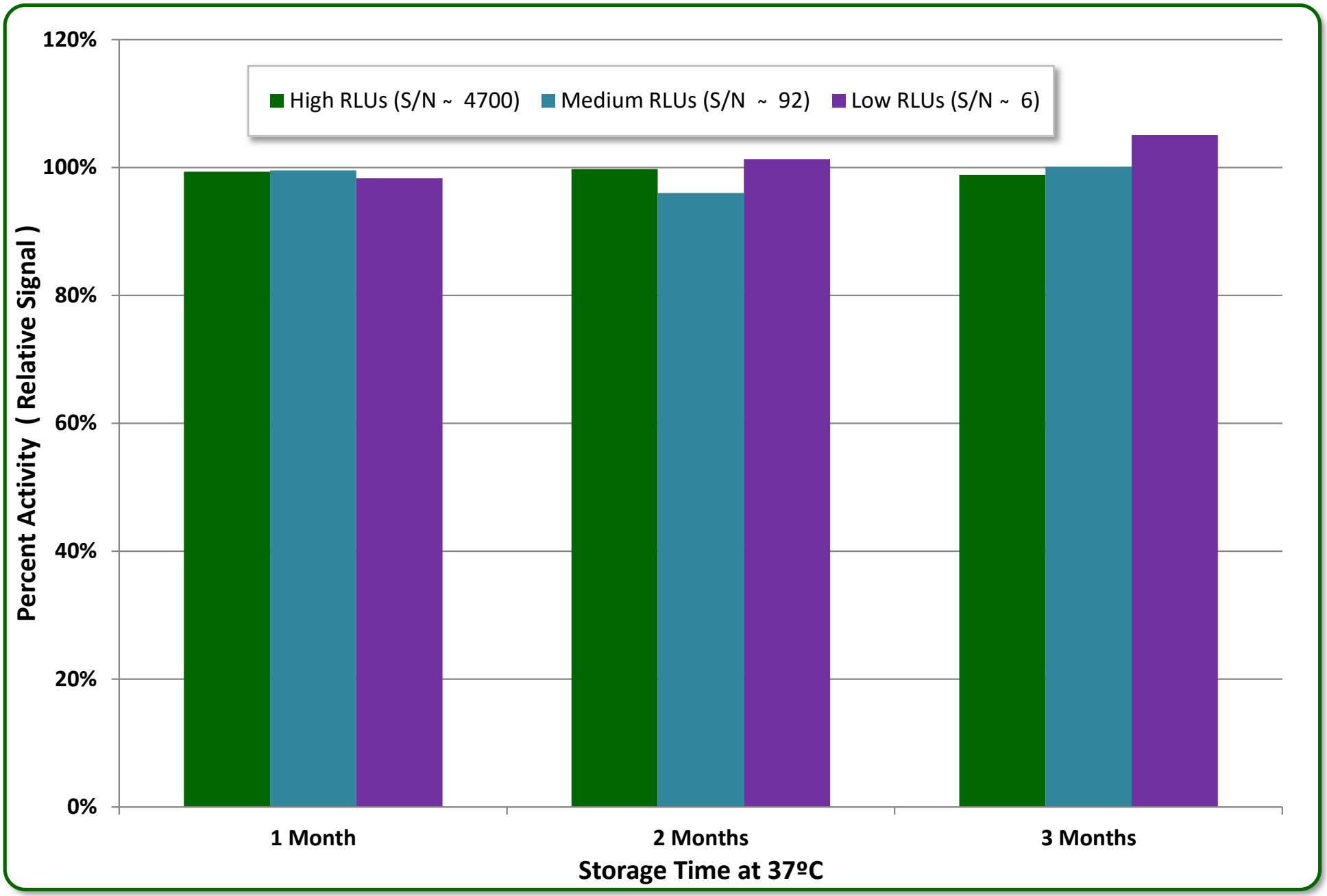


The Moss-CHEMI substrate can be diluted to adjust the signal level and reduce cost.

Stability of the working solution at room temperature

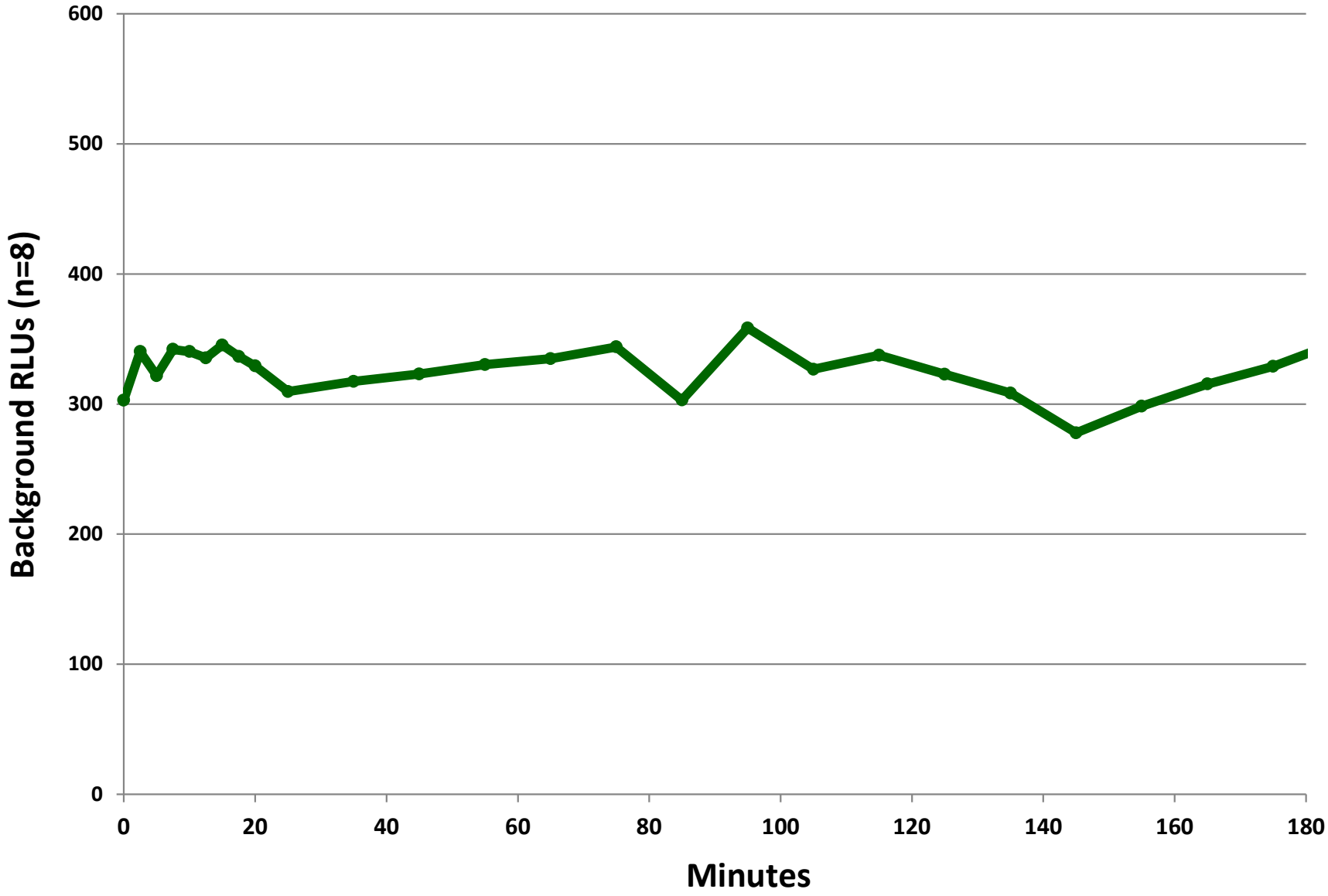


Accelerated Stability at 37ºC



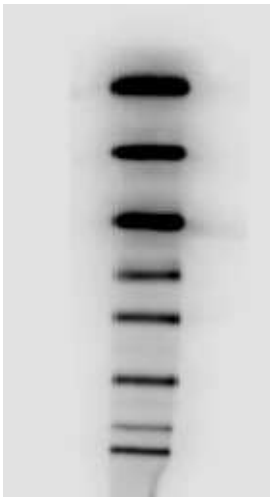
After 3 months at 37ºC, the substrate retains full reactivity.

Substrate Background Stability



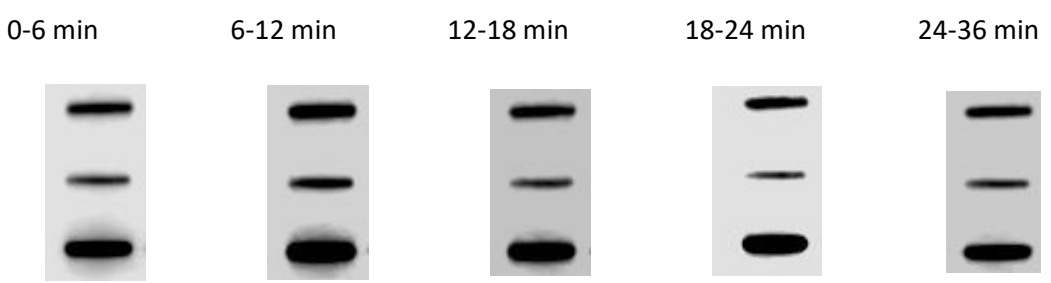
After addition to the wells, the substrate background is stable for more than two hours.

Western and Dot-Blot Data

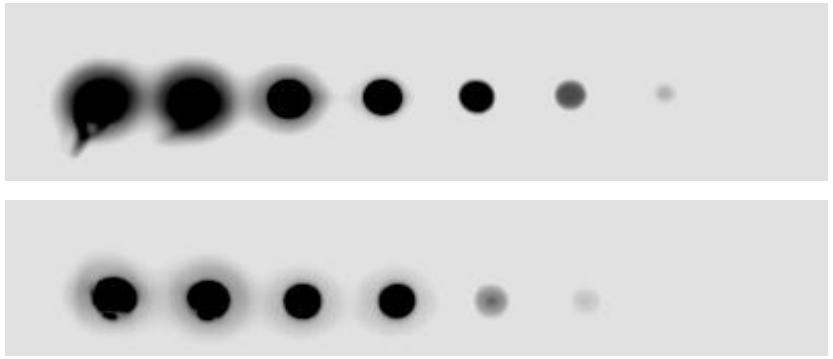


Western Blot detection of IgG-binding Proteins
(SuperSignal™ Molecular Weight Protein Ladder from Thermo)

Repeat Scans at 6-minute intervals on the Licor C-Digit



Dot Blot Dilution Series

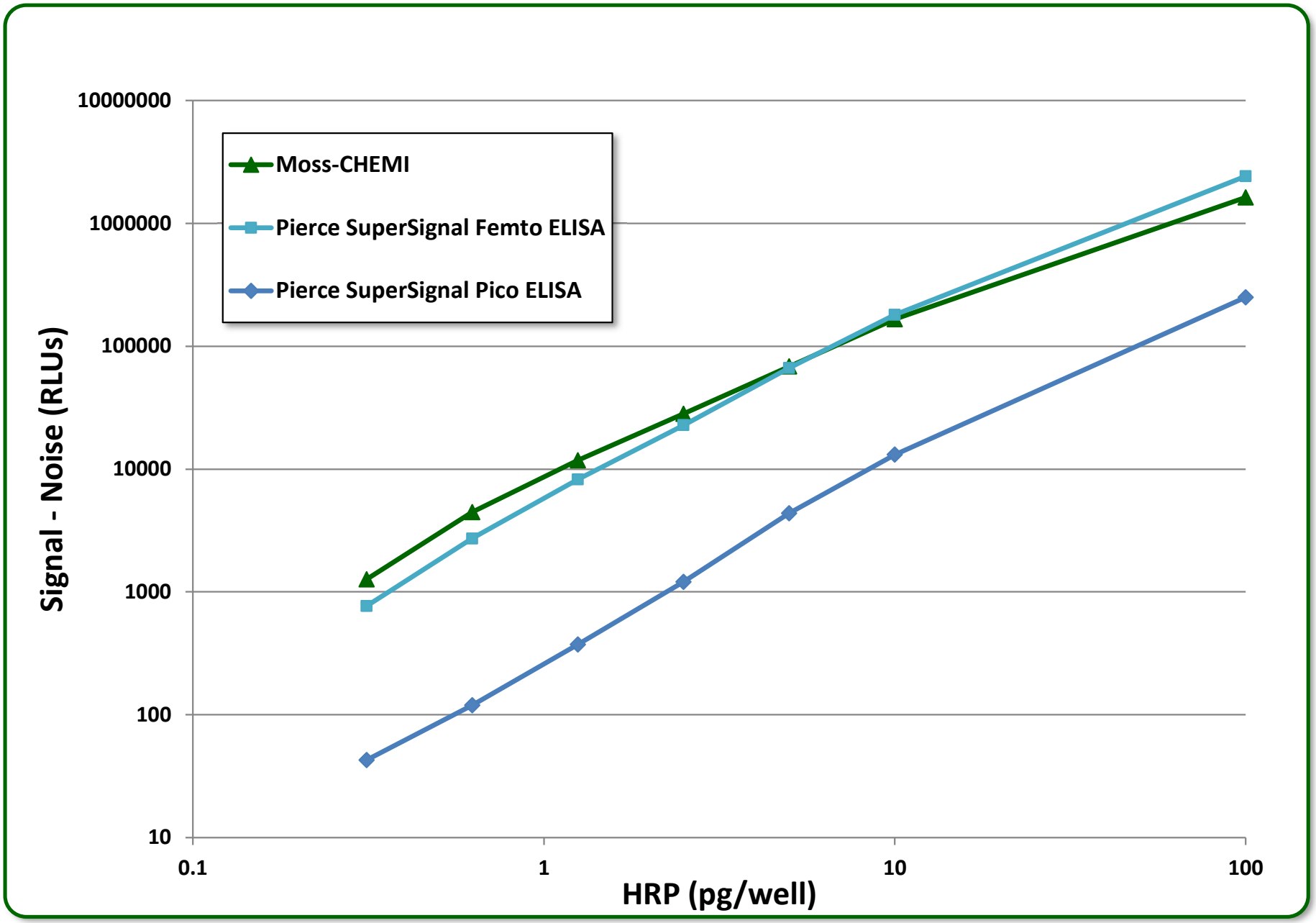


Moss Chemiluminescent Substrate

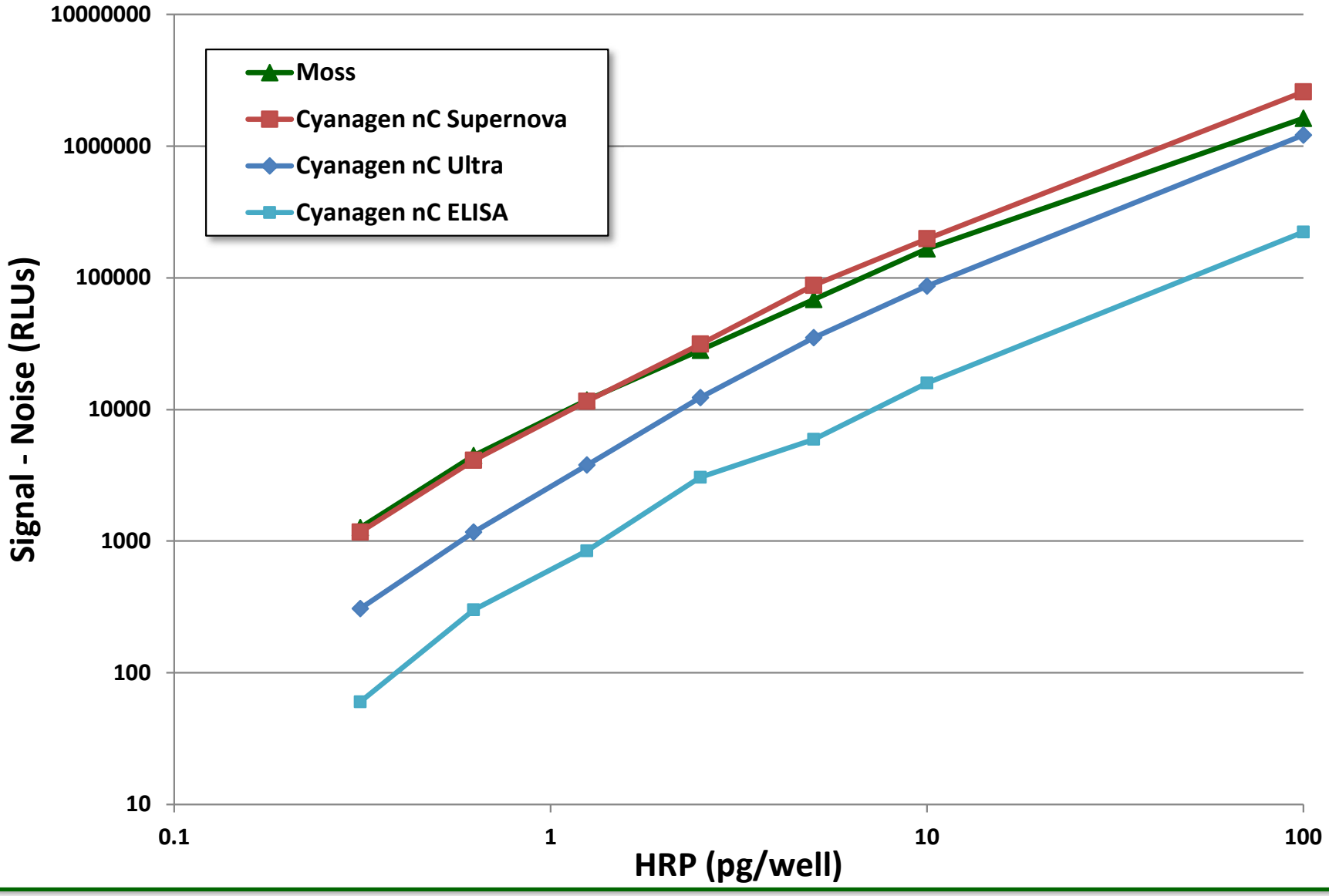
Competitor

Moss-CHEMI HRP substrate can provide high sensitivity detection in many blotting applications.

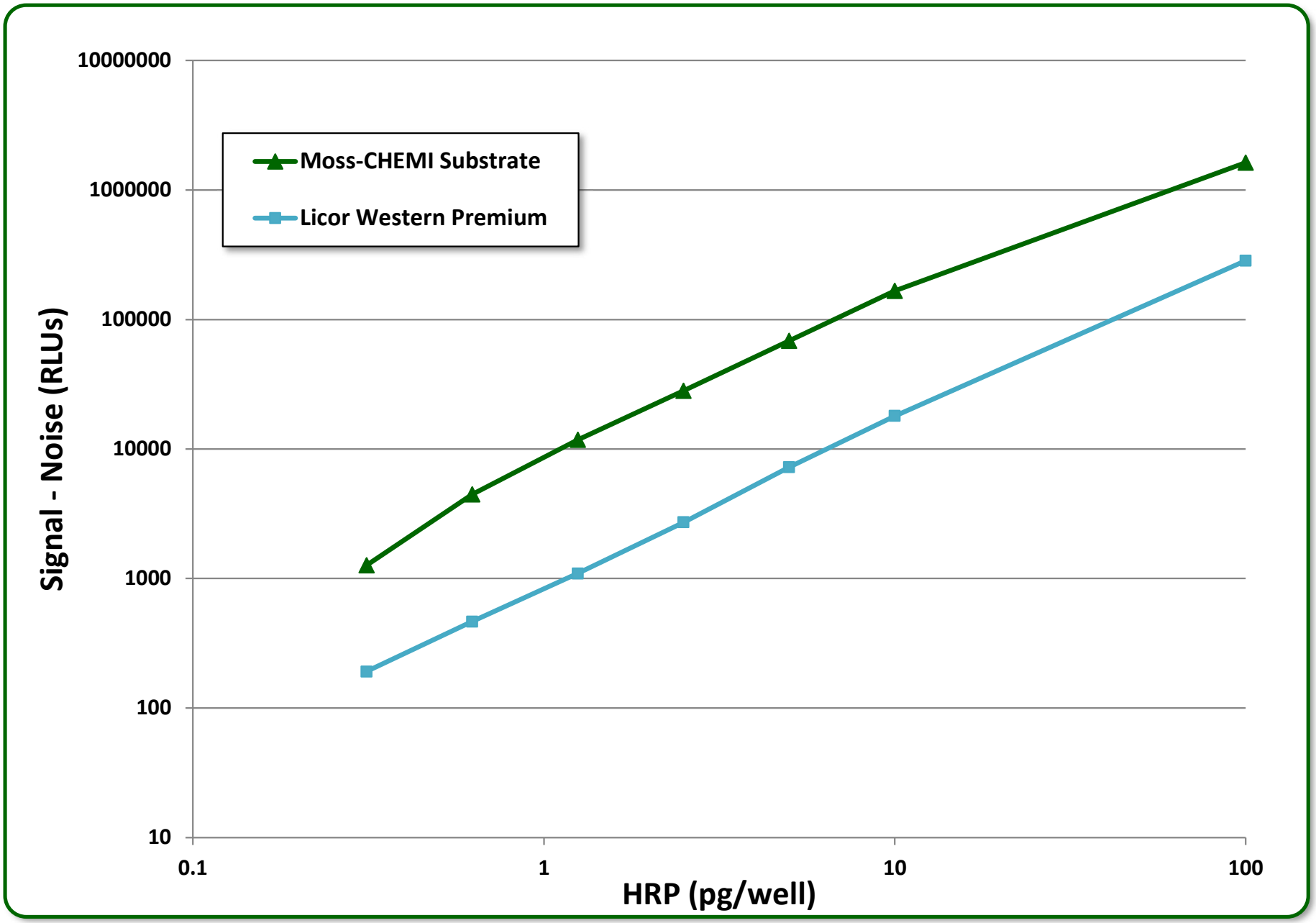
Moss-CHEMI vs Pierce



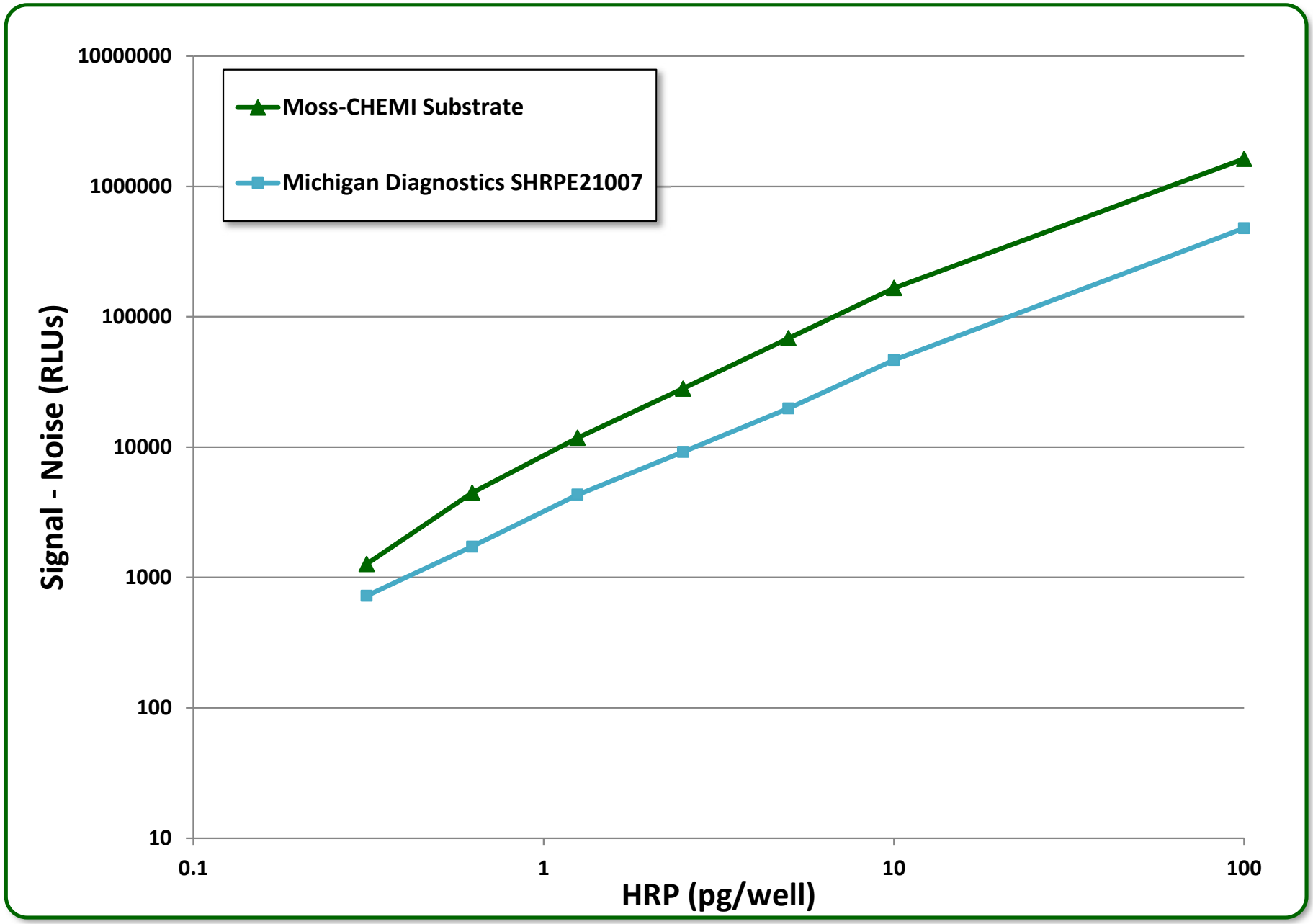
MOSS-CHEMI vs Cyanagen



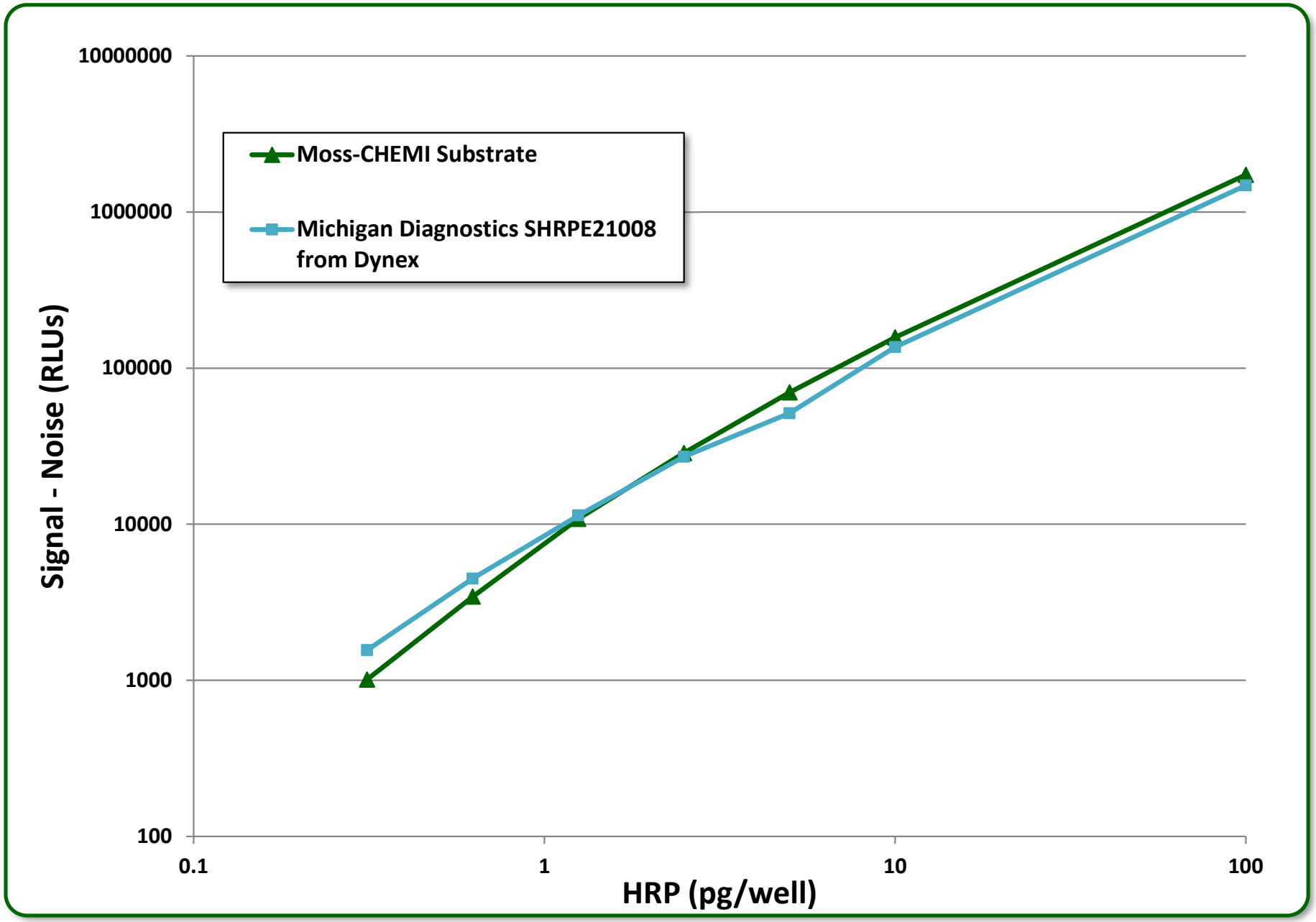
MOSS-CHEMI vs Licor Western Premium



MOSS-CHEMI vs Michigan Diagnostics SHRPE21007



MOSS-CHEMI vs Michigan Diagnostics SHRPE21008



Contact Information

Sales and Distribution:

Zach Downing
410-768-3442
zdowning@mossbio.com
www.mossbio.com

Technical:

Jim Lazar, Ph.D.
410-768-3442
jlazar@mossbio.com