

A no-wash, rapid FcRn binding immunoassay to guide the design and development of antibody therapeutics

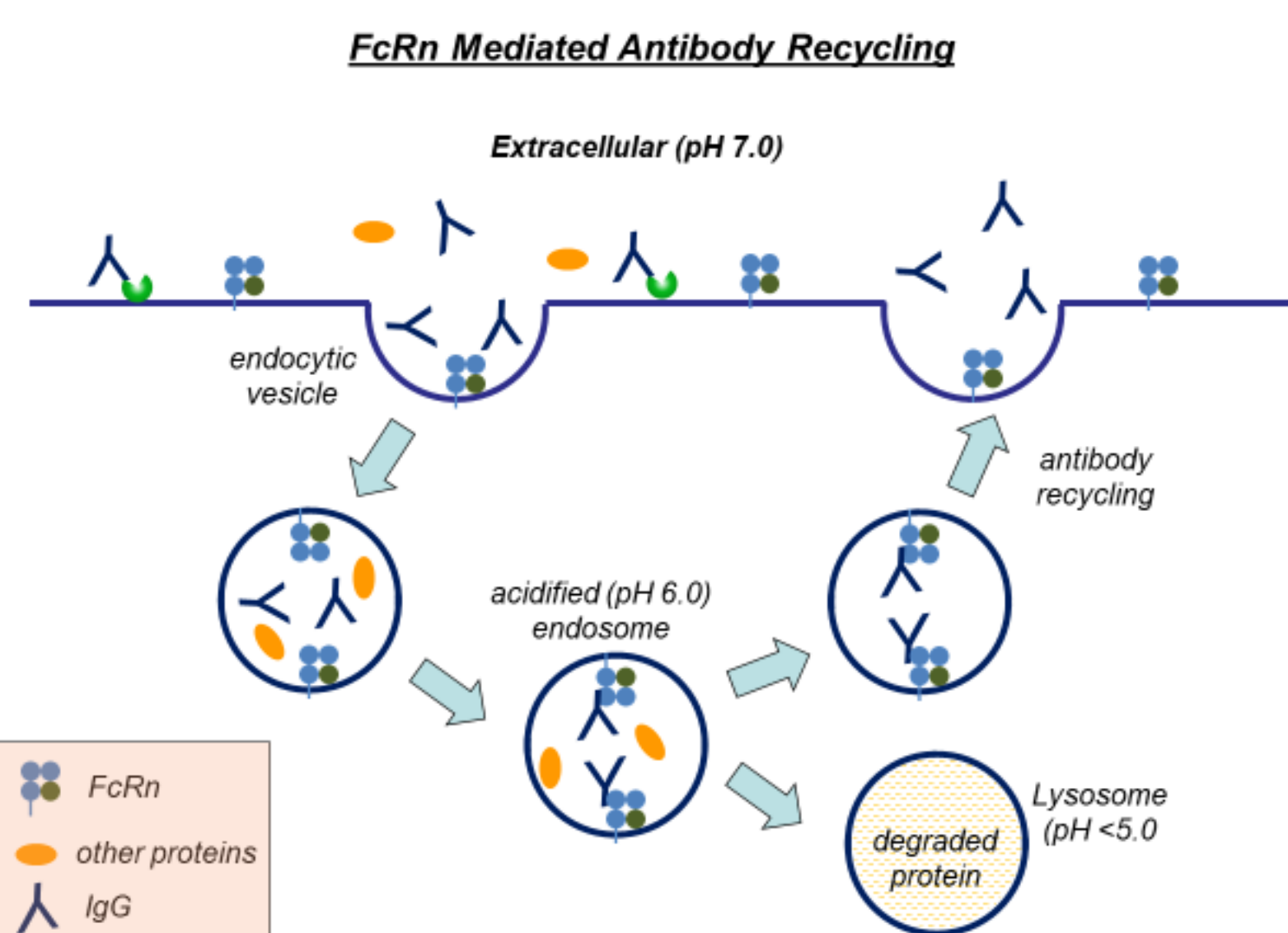
Nidhi Nath, Becky Godat, Rod Flemming, and Marjeta Urh

Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711



1. Introduction

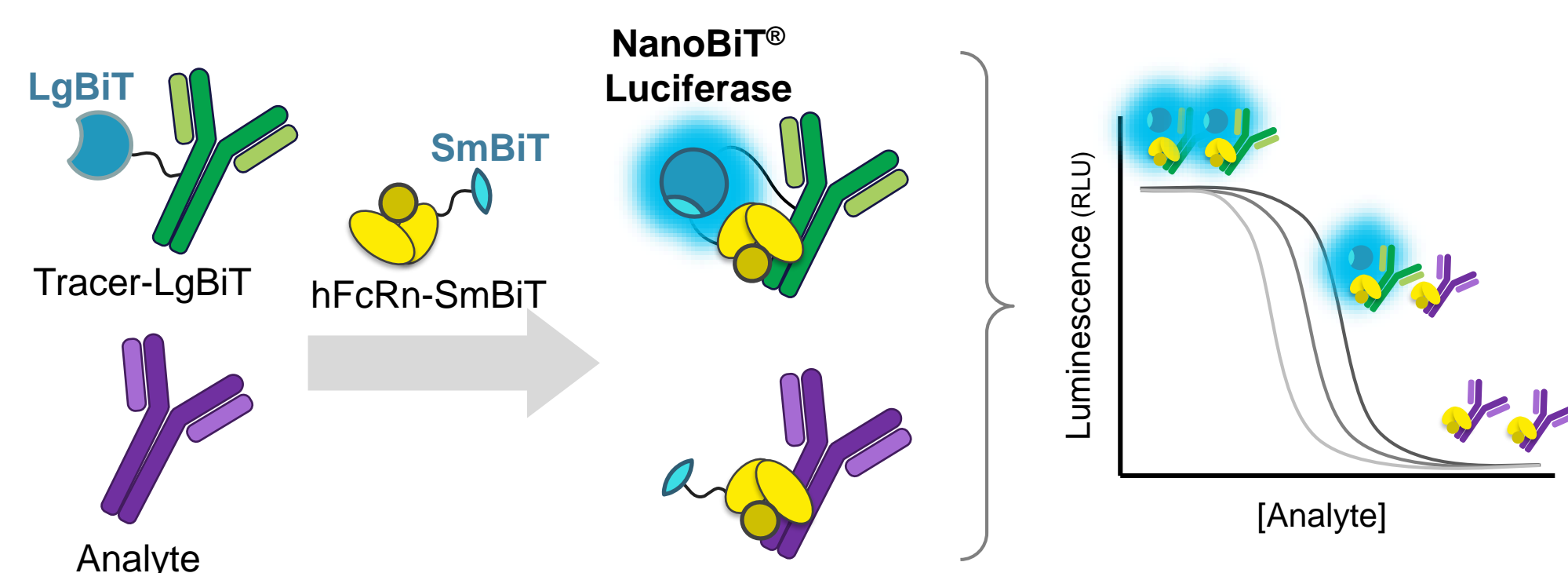
- The neonatal Fc Receptor (FcRn) is responsible for the relatively long half-life (~21 days) of antibodies in the body
- FcRn binds to the Fc region of an antibody (Ab), and is responsible for recycling antibodies or directing them for lysosomal degradation
- FcRn-Ab interaction can be modulated to increase or decrease antibody internalization and tune drug efficacy



Here, we present a solution-based (no immobilization step) homogeneous (no wash step) assay for measuring FcRn-Ab interaction using NanoBiT® technology.

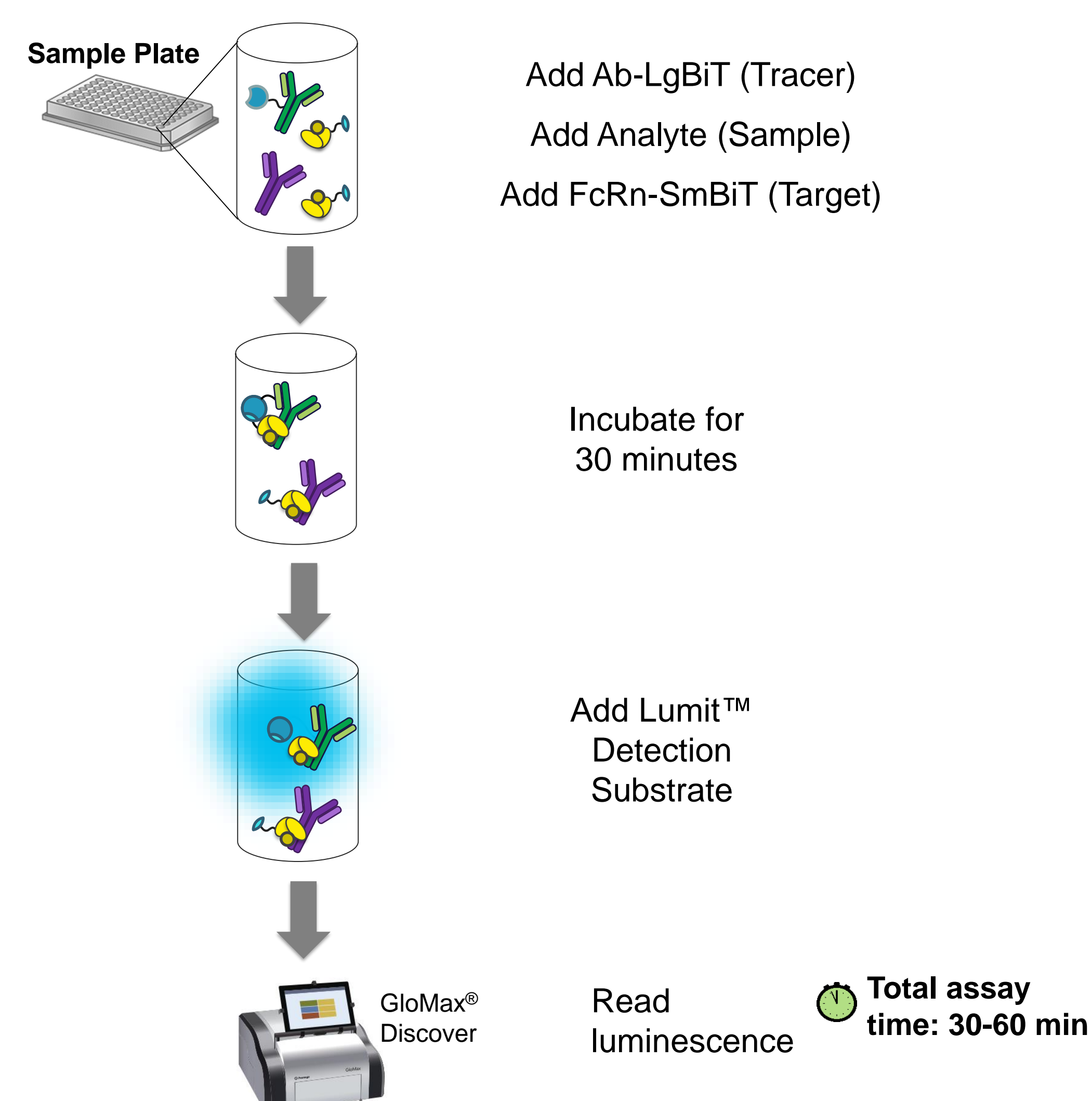
2. Lumit™ FcRn Binding Immunoassay

The Lumit™ FcRn Binding Immunoassay is a competition immunoassay based on NanoBiT® protein complementation technology

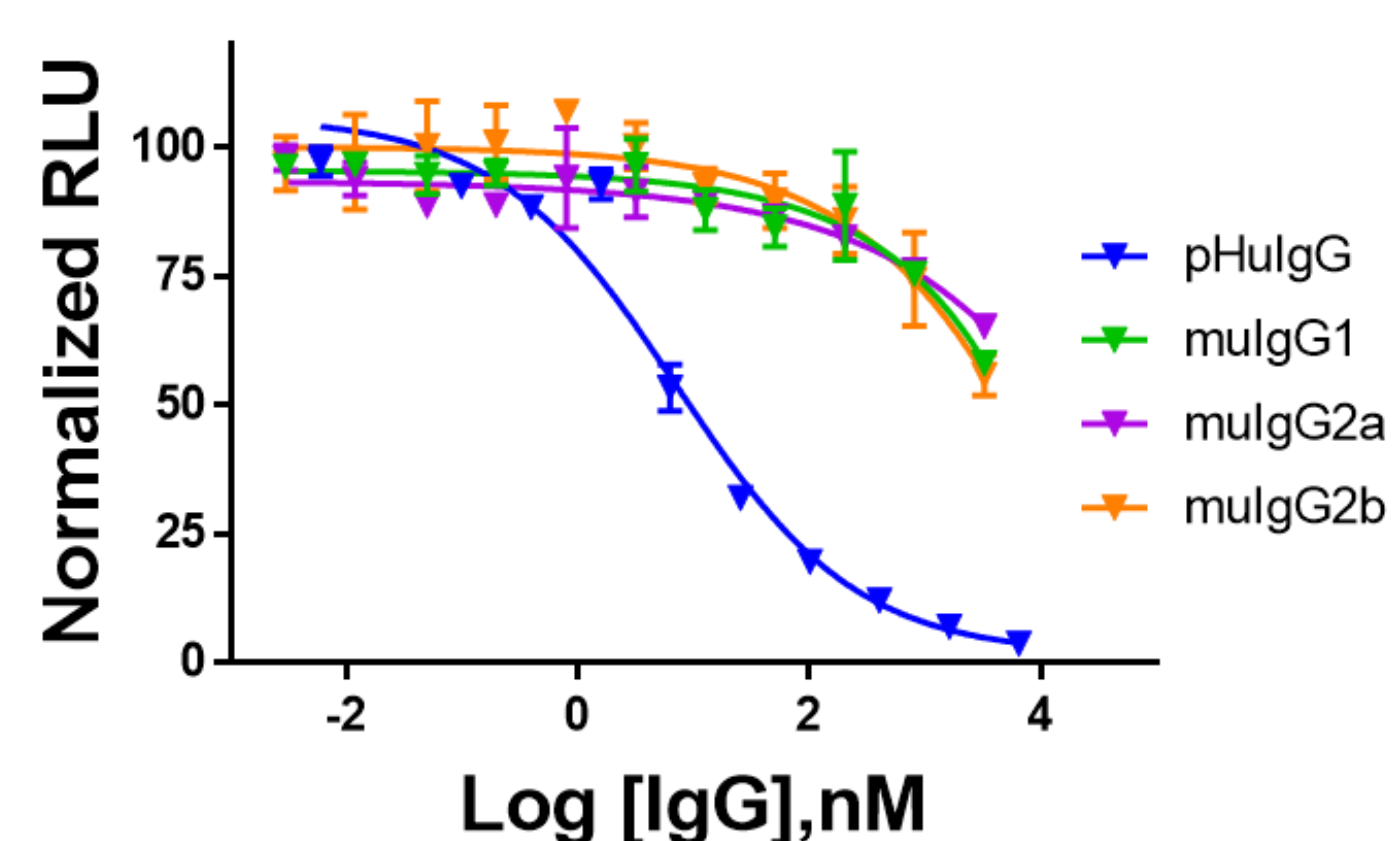
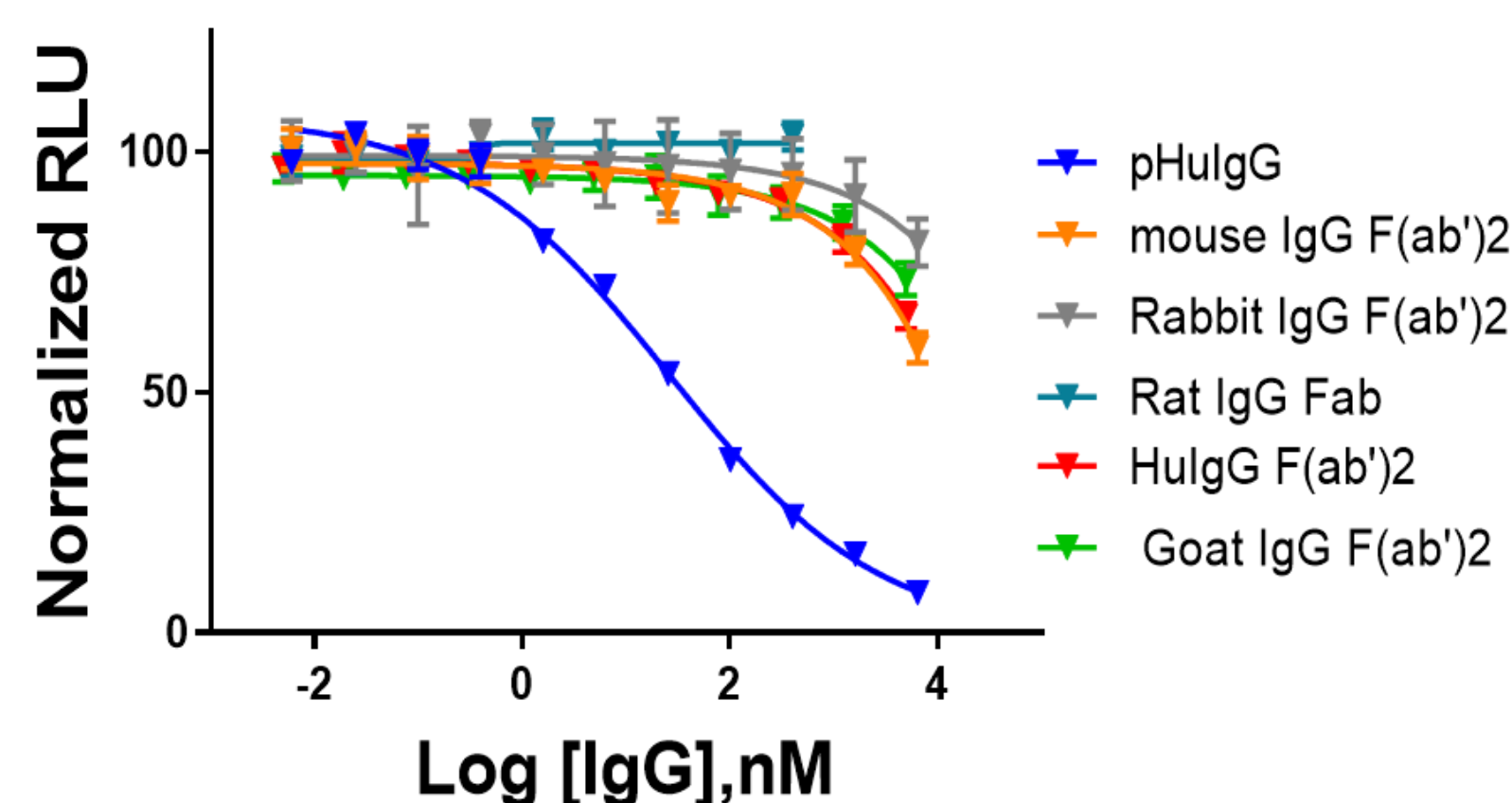


- Lumit™ immunoassays are solution-based; no experimental artifacts are introduced by receptor immobilization.
- Assays are homogeneous (add-mix-read) and require no washing.
- Luminescence based detection provides wide dynamic range and a large assay window.
- Assays are quick (30-60 min) and require low sample volume (10-25 µl).
- Compatible with 96/384-well plates for flexible throughput and automation.

3. Simple add-mix-read format



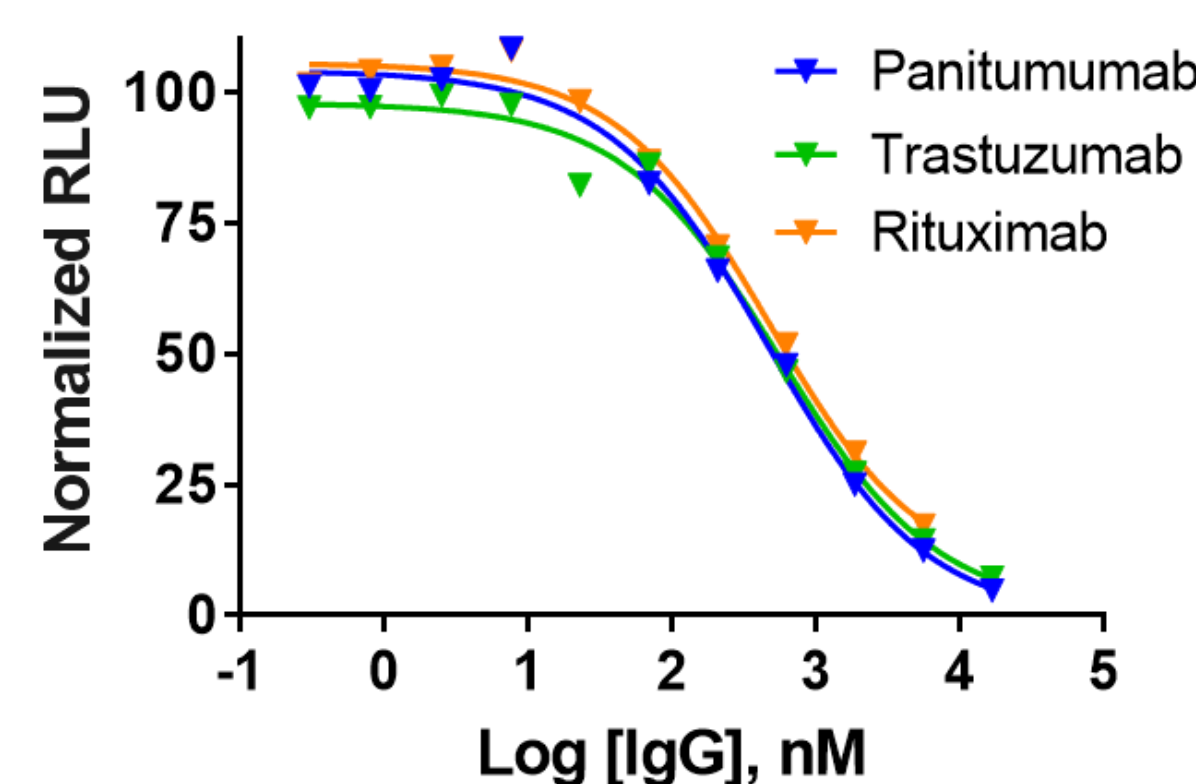
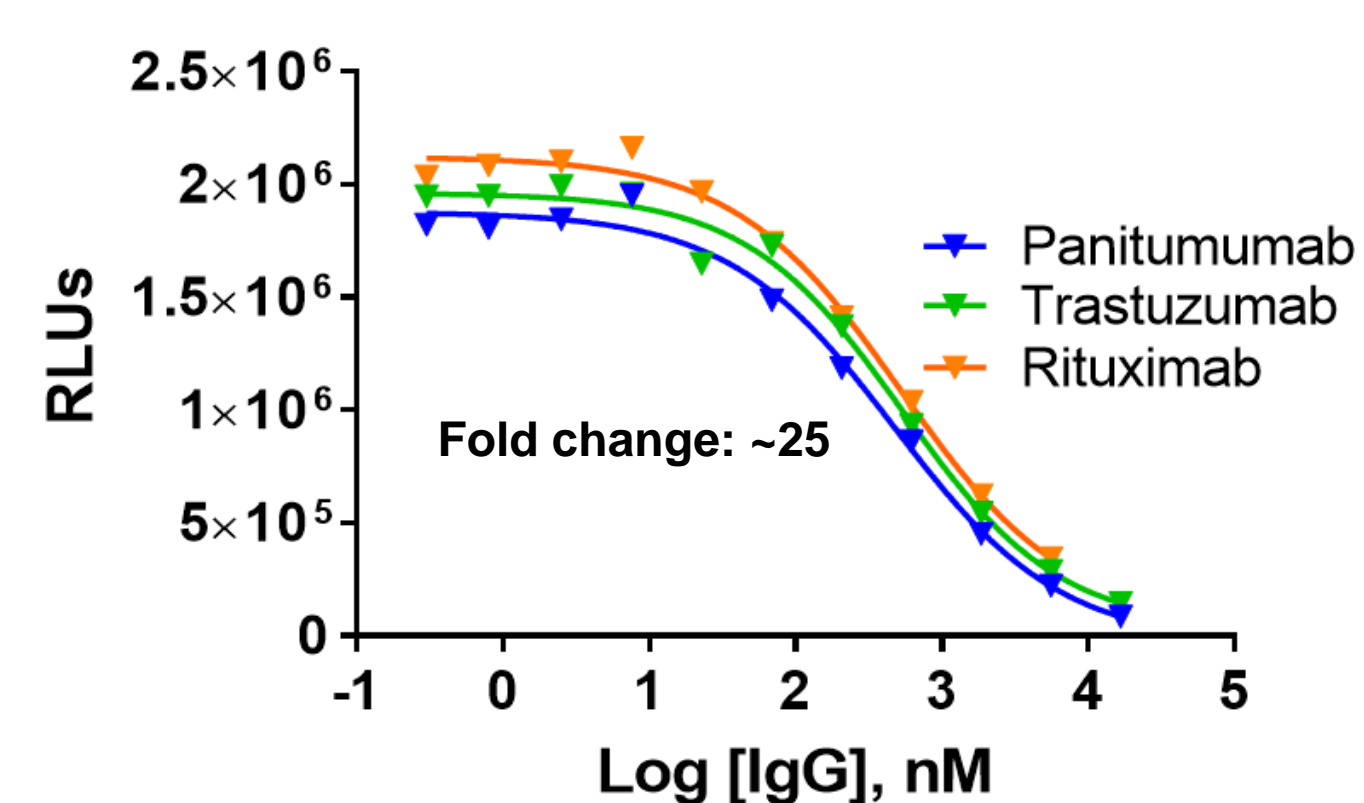
4. Assay specificity



Assay is highly specific:

- IgG Fab fragments with no Fc region show no binding
- Mouse antibodies show minimal binding activity

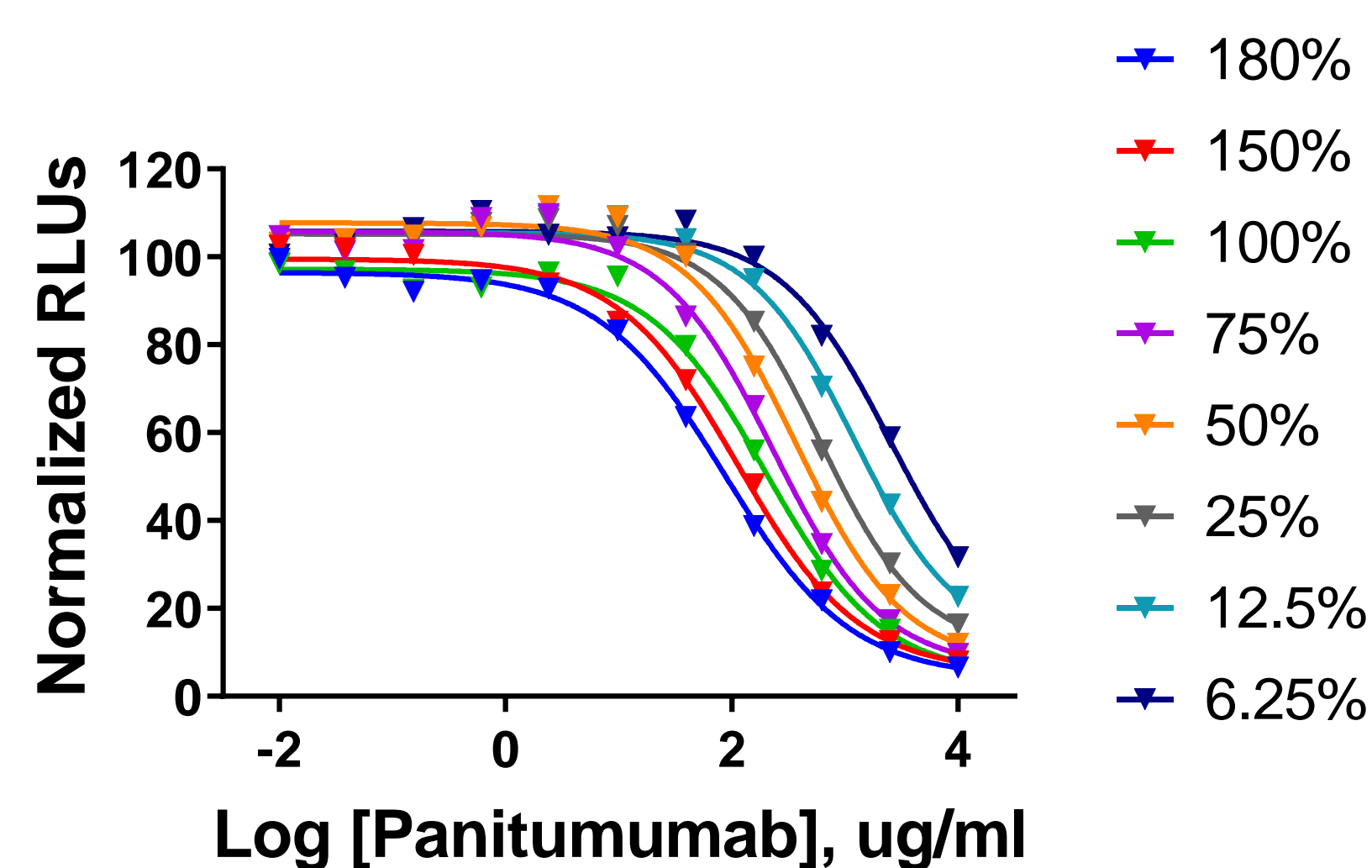
5. Assay use with therapeutic antibodies



Antibody	IC50 (nM)
Panitumumab	458.5
Trastuzumab	553.0
Rituximab	471.2

A good assay window is observed in both raw and normalized RLU with a variety of therapeutic antibodies.

6. Measurement of relative antibody potency



	180%	150%	100%	75%	50%	25%	12.5%	6.25%
IC50	86.66	113.9	200.4	231.1	369.9	604.7	1203	2553

Dose response curves for Panitumumab-FcRn binding corresponding to 180%, 150%, 100%, 75%, 50%, 25%, 12.5% and 6.25% of the nominal concentration, plotted versus nominal (100%) concentration.

7. Impact of oxidation on FcRn binding

Crystal structure of human IgG1-Fc

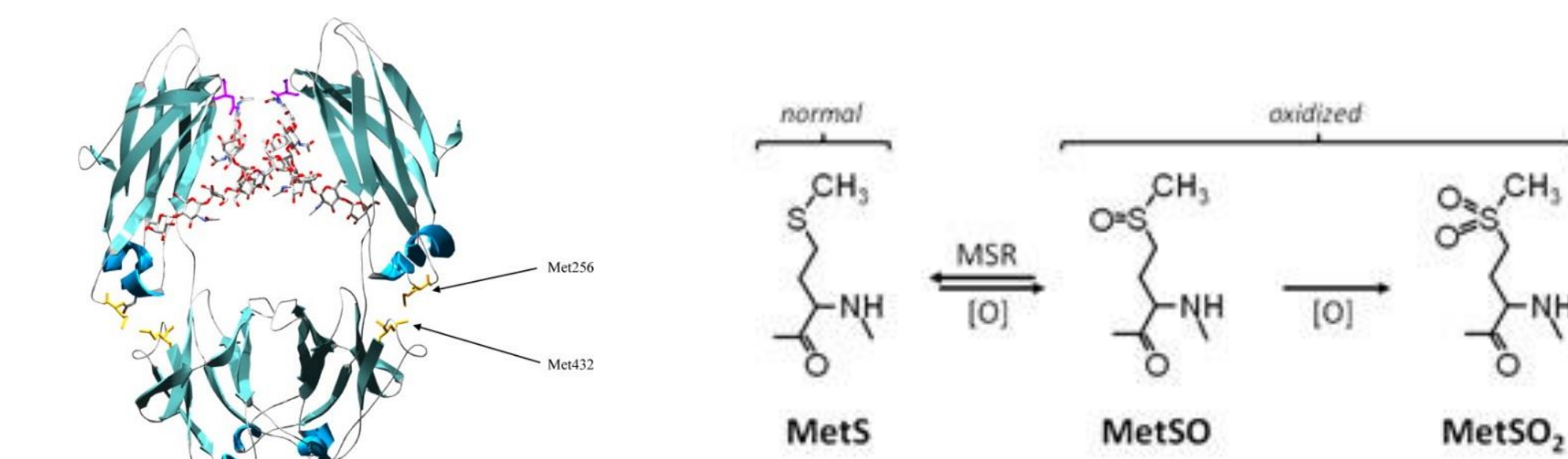
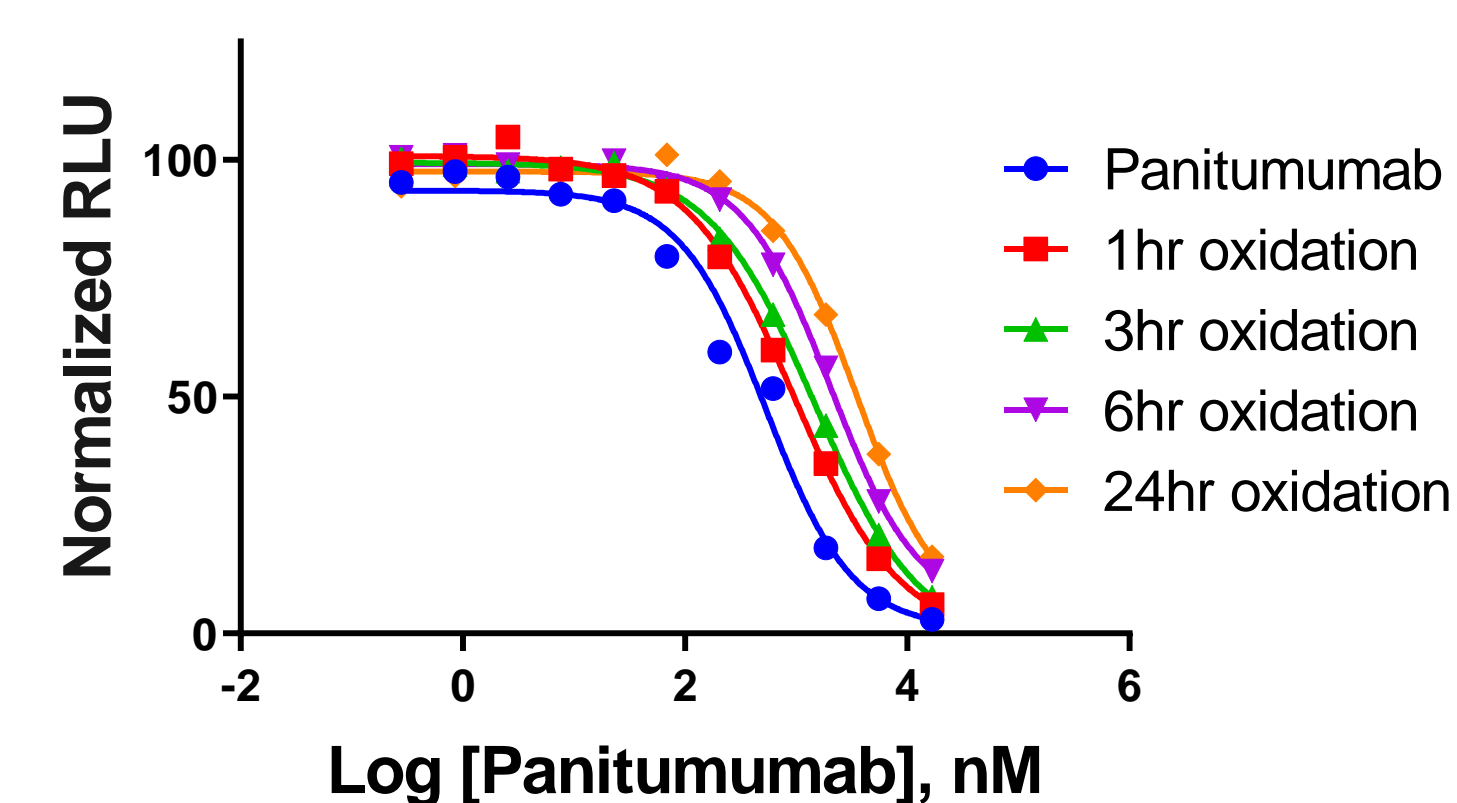


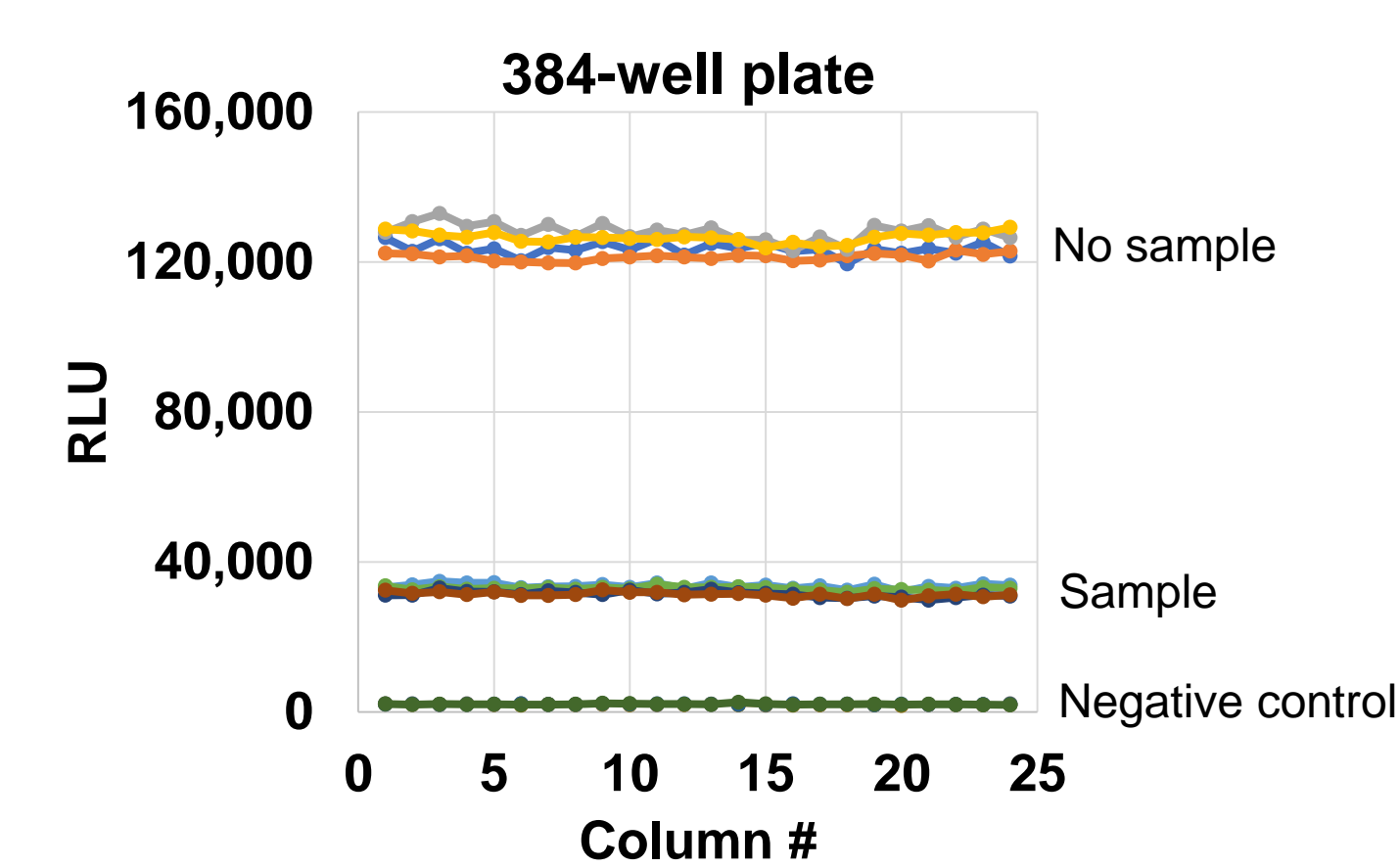
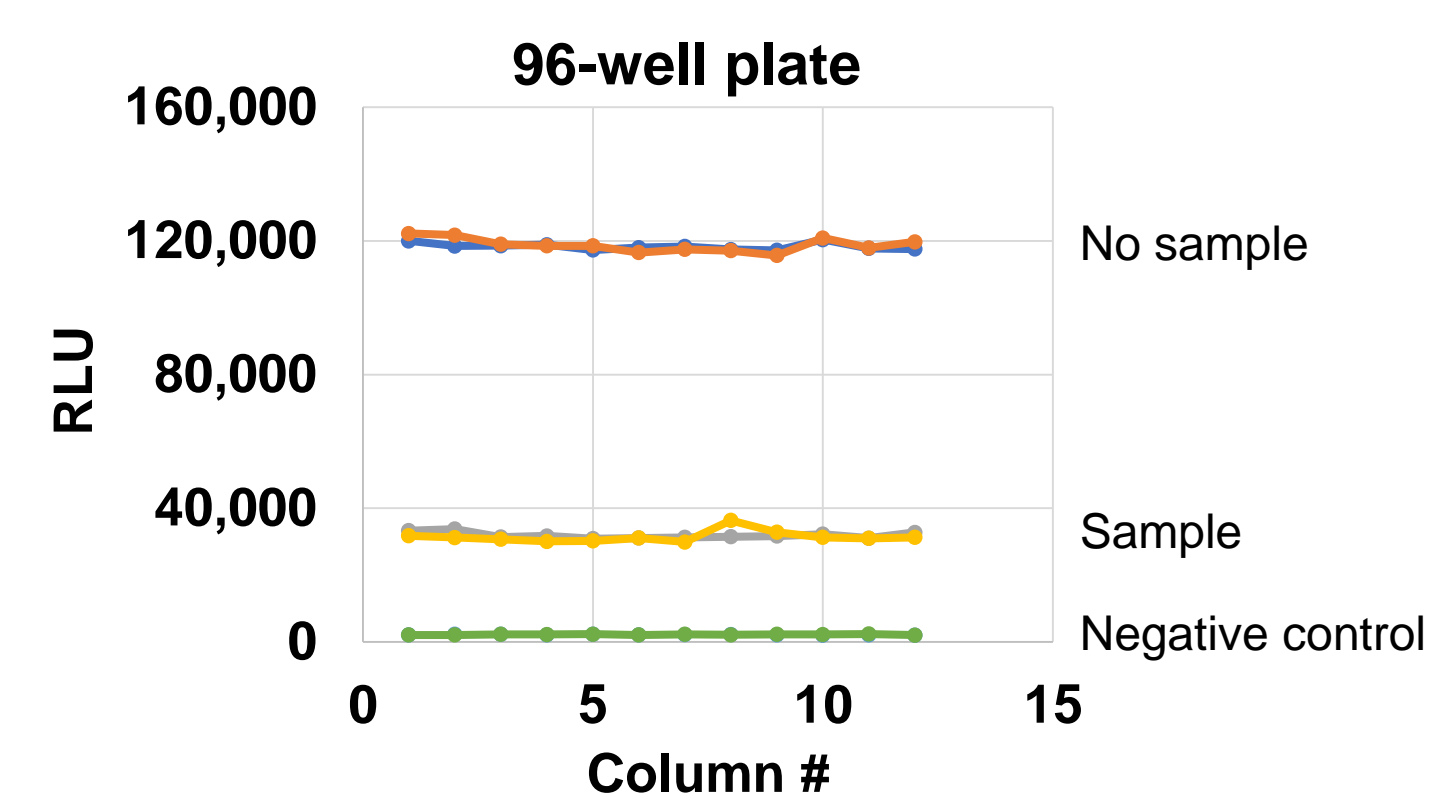
Image from Georjeen Gaza-Bulseco, et al. J Chromatography B 870(1), 55-62 (2008)



	Panitumumab	1hr oxidation	3hr oxidation	6hr oxidation	24hr oxidation
IC50	537.3	957.7	1478	2055	3510

- Panitumumab was incubated with 0.3% H₂O₂ for 1hr-24hr to oxidize methionine
- Oxidation resulted in significant reduction in affinities of antibodies towards FcRn, detectable in the assay

8. Suitable for high throughput screening



	Ave RLU (no sample)	CV	Z'
96 well plate	118,546	1.4	0.956
384 well plate	124,857	2.5	0.921

The assay can be scaled to 96 or 384 well plates with excellent CV and Z' values.

9. Summary

We have developed a rapid, solution-based, homogeneous immunoassay for FcRn-Ab binding that requires no immobilization steps or washes.

- Assay is quick (30-60 min)
- Requires low sample volume and can be automated in 96/384-well plates
- Luminescence detection provides a wide assay window
- Assay can track the oxidation state of antibodies