# Evaluating a No-Wash Rapid FcRn Immunoassay to Guide Development of Antibody Therapeutics

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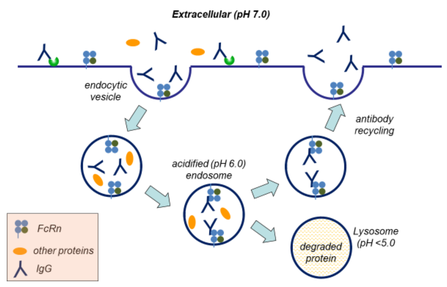
## 1. Introduction

Neonatal Fc Receptor (FcRn) is responsible for long half-life (~21 days) of antibodies in the body. FcRn binds to the Fc region of an antibody, 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.

Current methods for measuring FcRn-Ab interactions like SPR introduce artifacts due to immobilization steps and are multistep processes.

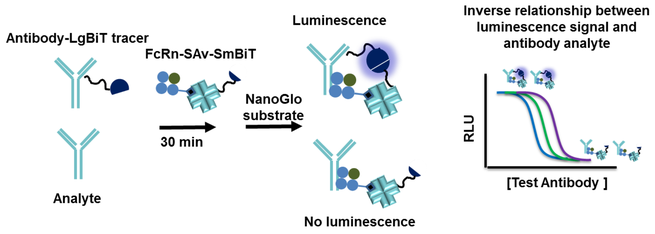
Here we present a solution-based (no-immobilization) homogeneous (no-wash) assay for FcRn-Ab interaction using NanoBiT technology.

**FcRn-Mediated Antibody Recycling**




## 2. NanoBiT FcRn Immunoassay

**NanoBiT FcRn Immunoassay is a competition immunoassay based on NanoBiT protein complementation technology**



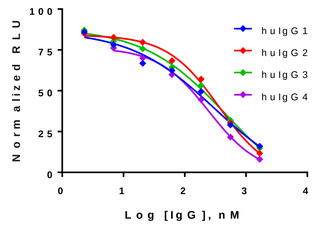

1. NanoBiT assays are solution-based (no immobilization) and may minimize artifacts introduced by immobilization.
2. Assays are homogeneous (add-and-read) and require no washing.
3. Luminescence based detection provides wide dynamic range and a large assay window.
4. Assays are quick (30min) and use low sample volume (10-20µl).
5. Use of 96/384-well white plates enables flexible throughput and automation capabilities.

## 3. Simple Add-Mix-Read Format

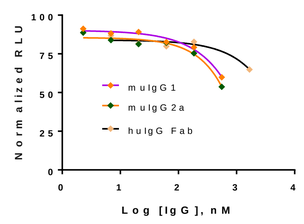
1. Add 25µl of antibody sample at pH 6.0
2. Add 25µl of hIgG1-LgBiT tracer + 50µl of FcRn-SmBiT
3. Incubate 30min
4. Add 25µl FcRn Glo reagent
5. Read Luminescence

## 4. Measuring Dissociation Constants (Kd) of Panel of Human and Mouse Antibodies

**Competition Assay (hFcRn-hIgG)**



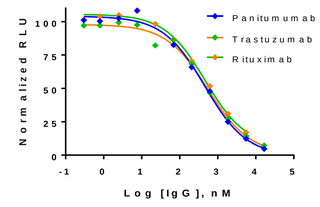

**Competition Assay (hFcRn-mIgG)**



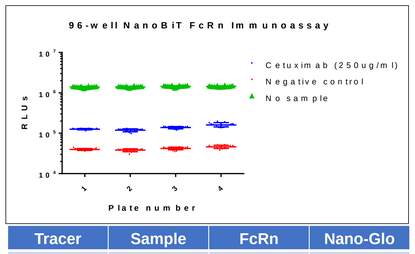

**Assay is highly specific:**

- Minimal binding of mouse antibodies
- Human IgG Fab fragment with no Fc region shows no binding

**hFcRn Competition Assay (normalized)**

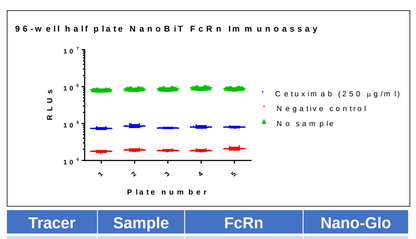



## 5. Assay is Reproducible and Scalable for High-Throughput Screening




| Tracer | Sample | FcRn | Nano-Glo |
|---|---|---|---|
| 25µl | 25µl | 50µl | 25µl |

| Plate | Z` |
|---|---|
| 1 | 0.86 |
| 2 | 0.81 |
| 3 | 0.71 |
| 4 | 0.70 |

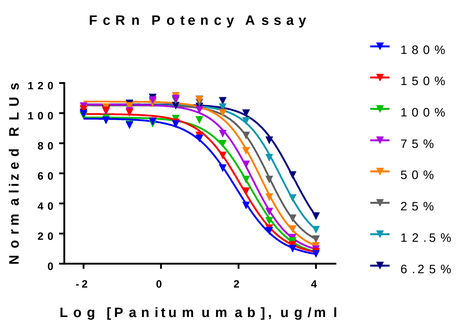



| Tracer | Sample | FcRn | Nano-Glo |
|---|---|---|---|
| 10µl | 10µl | 20µl | 10µl |

| Plate | Z` |
|---|---|
| 1 | 0.91 |
| 2 | 0.81 |
| 3 | 0.82 |
| 4 | 0.78 |
| 5 | 0.85 |

Z' factor values obtained in both 96- and 96-half well plates. Assay was performed using 10µl and 25µl sample volumes and add-mix-read format shown in Panel 3. Green dots are the maximum RLUs in absence of any sample, Red dots are the background signal and blue dots are the RLUs in presence of 250µg/ml of Cetuximab.

## 6. Measuring Relative Potencies of Antibodies with Dose Response Curves




| | 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 values

## 7. Impact of Oxidation on FcRn Binding

**Crystal structure of human IgG1–Fc**

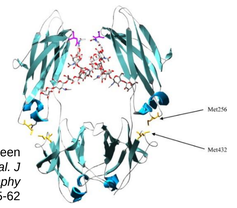
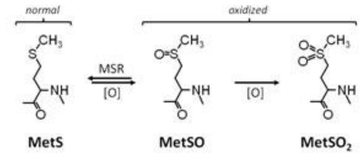
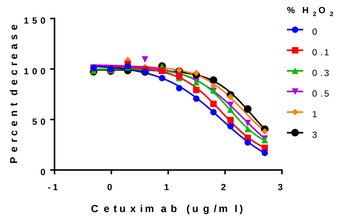


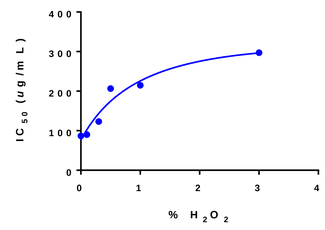

Image from Georgeen Gaza-Bulseco, *et al. J* (2008) *Chromatography* B 870(1), 55-62




**Dose-response curve of Cetuximab after forced oxidation with $H_2O_2$**




**$IC_{50}$ of Cetuximab after forced oxidation with $H_2O_2$**

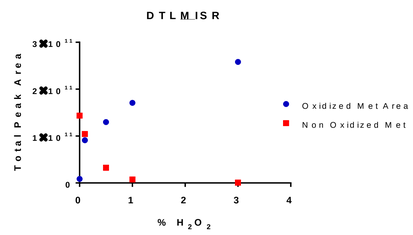


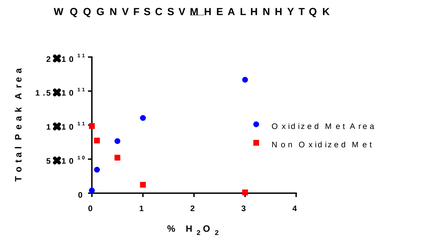

| | 0 | 0.1 | 0.3 | 0.5 | 1 | 3 |
|---|---|---|---|---|---|---|
| IC50 | 85.68 | 88.81 | 133.2 | 201 | 197.9 | 252.8 |

## 8. Oxidation of Cetuximab Measured Using LC-MS

**LC-MS/MS workflow**

**Antibodies**
↓
**Oxidation**
↓
**Denaturation / reduction / alkylation**
↓
**Trypsin digest**
↓
**MS**




Oxidation of Cetuximab, measured using LC-MS, correlates with changes in IC50 values as measured using NanoBiT FcRn Immunoassay

## 9. Conclusions

Current methods for measuring FcRn-Ab interactions like SPR introduce artifacts due to immobilization steps and are multistep processes.

To address this limitation, we have developed a rapid, solution-based, homogeneous assay for FcRn-Ab binding that requires no immobilization or washes.

- Assay only takes 30min
- Requires low sample volume; 96/384-well plate automation friendly
- Luminescence detection provides a wide assay window
- Assay can be used to track the oxidation state of the antibodies

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