Fc Effector Bioassays for Rapid and Quantitative Measurement of ADCC and ADCP Mechanisms of Action

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1. Introduction

Drug developers and regulatory authorities recognize antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) as important mechanisms of action (MOA) of therapeutic antibodies. Traditional ADCC and ADCP bioassays use primary cells, which are labor intensive and highly variable. Less variable, easy-to-use and consistent analysis of these important MOA is needed in drug development programs.

To meet this need, we have developed a suite of functional cell-based Fc Effector reporter bioassays for the following receptors:

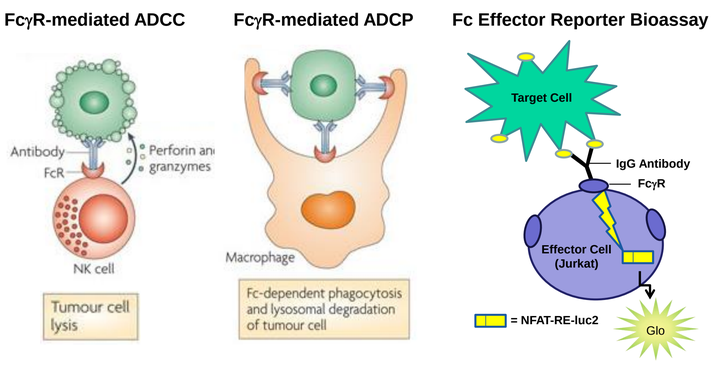
- **Human FcγRIIIa (V158 and F158 variants)**
- **Human FcγRIIa (H131 and R131 variants)**
- **Human FcγRI**
- **Mouse FcγRIV* & FcγRIII***

Each bioassay is provided in "thaw-and-use" format for a rapid and convenient workflow and further reduction in assay variability. In qualification studies according to ICH guidelines, the bioassays show specificity, accuracy, precision, and linearity enabling their use in antibody screening, characterization, stability and potency studies.

*Mouse Fc Effector Bioassay data are not shown here (please enquire)

2. FcγR Reporter Bioassay Concept, Format and Workflow

Surrogate Measure of In Vivo Biology



Bioassay Components:

- Effector Cells: Jurkat T cells genetically engineered with the appropriate FcγR and an NFAT-response element that drives luciferase expression (NFAT-RE-luc2)
- Target Cells: Customer-defined*
- IgG Antibody: Customer-defined*

  *CD20+ Raji or WIL2-S cells and an anti-CD20 Ab are available as a positive control.

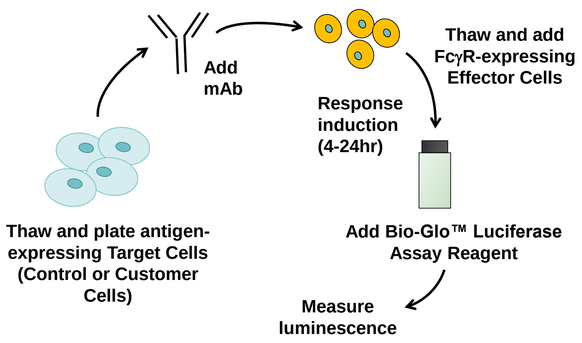
Bioassay Kit Formats:

- Core Kits:

  Effector Cells, Medium, Low IgG Serum, Bio-Glo™ Luciferase Reagent
- Complete Kits:

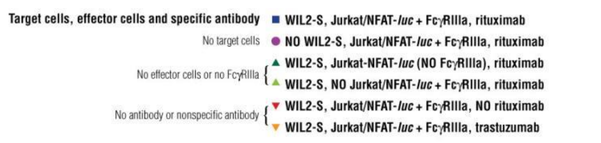
  Core Kit components listed above, Target Cells (CD20+), Control Ab, Anti-CD20

Rapid & Convenient Workflow



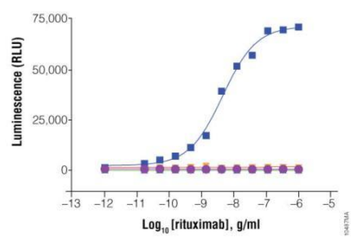
3. FcγR Reporter Bioassays are Specific

Rituximab: chimeric anti-CD20 mAb (expressed on WIL2-S cells)
Trastuzumab: anti-Her2/neu mAb (not expressed on WIL2-S cells)



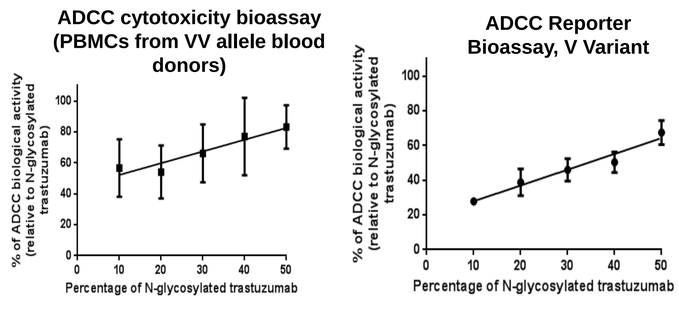
Assay signal is dependent on:

- Target cells expressing Ab-targeted antigen
- Effector cells expressing FcγR
- Antigen-specific IgG antibody

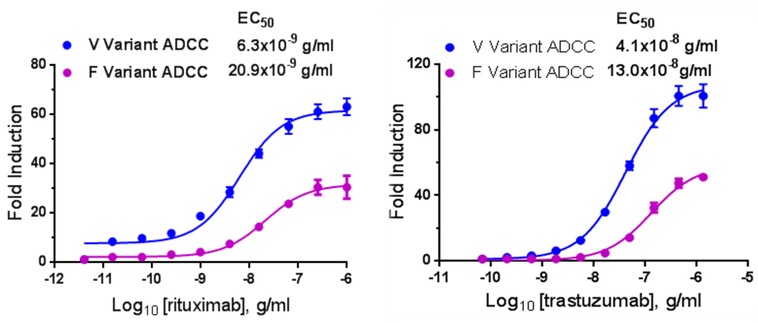


Variations of the ADCC Reporter Bioassay (FcγRIIIa-V158) were used to measure the Fc effector function of either Rituximab or Trastuzumab to demonstrate assay specificity. Similar results were obtained using all of the FcγR Reporter Bioassays referenced in panel 1 (data not shown).

4. FcγRIIIa-V158 and -F158 Reporter Bioassays Measure Expected Potency



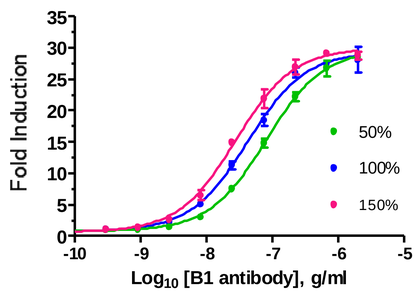
Antibody (trastuzumab) potency was measured using a primary cell-based ADCC assay (left panel) and the ADCC Reporter Bioassay (FcγRIIIa-V158, right panel). The surrogate ADCC Reporter Bioassay shows similar potency but much less variability (as indicated by the smaller error bars).



Rituximab and trastuzumab potency was measured using the FcγRIIIa-V and -F Reporter Bioassays. The data are consistent classic primary cell-based ADCC assays using PBMCs from donors with FcγRIIIa V158 and F158 alleles.

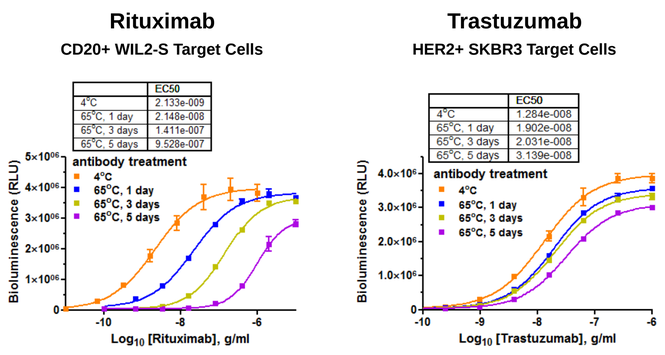
5. FcγR Reporter Bioassays Measure Antibody Potency and Stability

Potency Determination



The **relative potency** of anti-CD20 Ab preparations were measured using the ADCC Reporter Bioassay (FcγRIIIa-V158). Excellent accuracy, precision, and linearity in the range of 50-150% relative potency (per ICH guidelines) was demonstrated (shown here and data not shown).

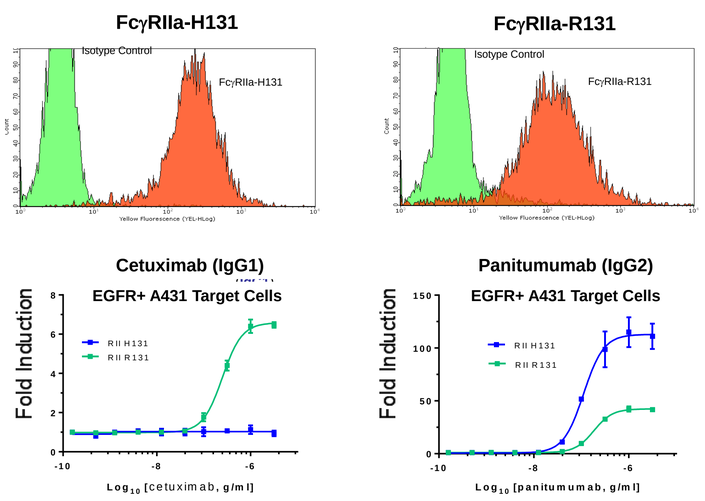
Stability Indication



The ADCC Reporter Bioassay (FcγRIIIa-V158) was used in a stability study of Rituximab and Trastuzumab following heat denaturation at 65°C for the indicated number of days.

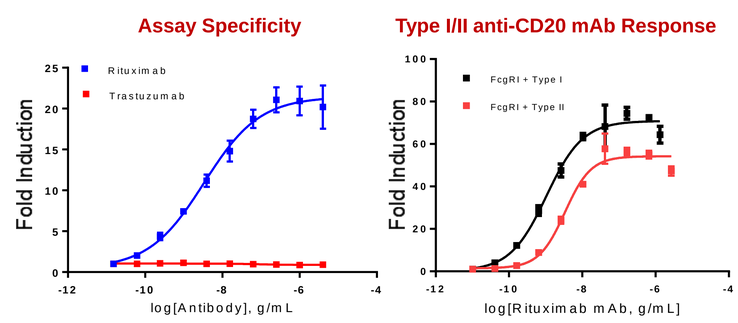
Similar measurements of antibody potency and stability were achieved using all the FcγR Reporter Bioassays references in panel 1 (data not shown).

6. FcγRIIa-H131 and -R131 Reporter Bioassays Reveal Antibody Mechanism of Action



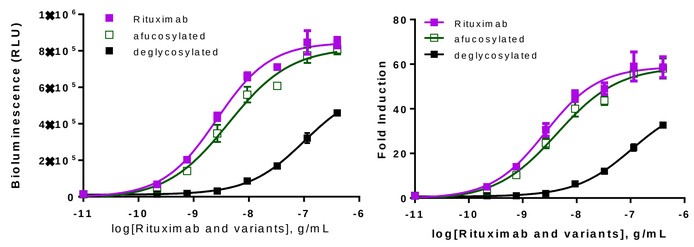
FcγRIIa-H131 and -R131 cell surface expression was measured by FACS analysis and shown to be similar (top panels). FcγRIIa-R131, but not -H131, is activated in response to Cetuximab (IgG1). Conversely, both FcγRIIa-H131 and -R131 are activated in response to Panitumumab (IgG2), with -H131 mediating a stronger response.

7. FcγRI Reporter Bioassay Specificity and Sensitivity to Antibody Glycosylation



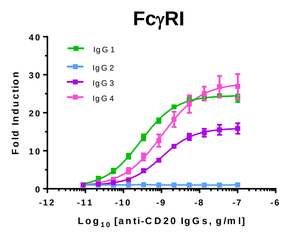
CD20+ Raji Target Cells were assayed with Rituximab (anti-CD20) or Trastuzumab (anti-Her2/neu). Rituximab, but not Trastuzumab, induced a robust response, as expected (left panel). In addition, Raji cells were incubated with either Type I or Type II anti-CD20 mAbs, and Type I elicited a stronger response (right panel).

Sensitivity to Antibody Deglycosylation

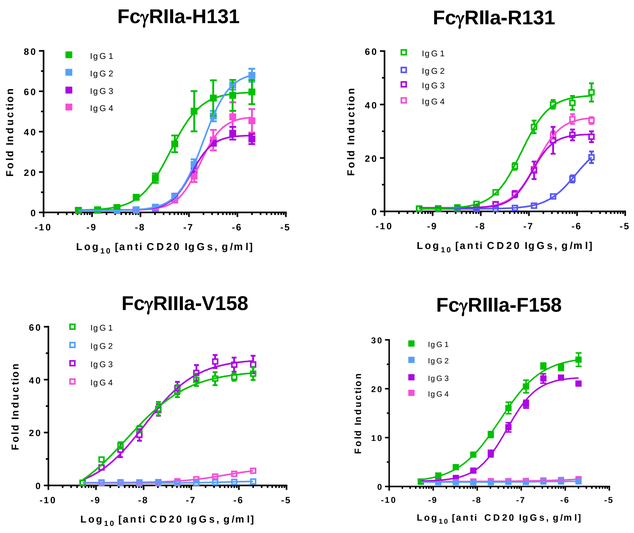


Raji Target Cells were assayed with Rituximab preparations that were either untreated, afucosylated or deglycosylated, as indicated. Deglycosylated antibody showed a significant decrease in bioluminescence (left panel) and fold induction (right panel), as expected.

8. FcγR Bioassays Show Appropriate IgG Isotype Bioactivities



A panel of FcγR Reporter Bioassays was used to measure the Fc effector activity of human anti-CD20 antibodies of different IgG isotypes. In all experiments CD20+ Raji Target Cells were used.



EC50 (g/ml)	FcγRI	FcγRIIa-H131	FcγRIIa-R131	FcγRIIIa-F158	FcγRIIIa-V158
IgG1	2.8e-010	4.15E-08	7.03E-08	3.34E-08	4.50E-09
IgG2	NB	1.98E-07	>9.60E-07	NB	NB
IgG3	1.0E-09	1.14E-07	1.18E-07	4.75E-08	1.07E-08
IgG4	~ 1.0e-09	1.71E-07	1.45E-07	NB	NB

9. Conclusions

Drug developers are rapidly adopting Fc effector function reporter-based bioassays to measure antibody Fc functions such as ADCC and ADCP activity during the development of therapeutic antibodies. Here we show application of a suite of FcγR Reporter Bioassays to elucidate and characterize antibody MOA. These bioassays provide the following:

Biologically relevant measurement of antibody MOA

- Specific FcγR-induced expression of NFAT-RE driven luciferase
- Reporter-based bioassay technology that is now well-established and understood to represent proper antibody mechanism of action

Consistent and reliable measure of antibody activity

- Demonstrated precision, accuracy, reproducibility, robustness
- "Thaw-and-use" cell format, no cell culture required

Easy-to-implement

- Multiple product formats meet diverse workflows; commercial kits include necessary reagents
- Rapid protocol for standard 96-well plate format

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