Binding to Bridging: Complete Suite of FcγR Assay Tools Accelerates Antibody Therapeutic Development

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1. Introduction

Therapeutic antibodies and Fc fusion proteins are effective against a variety of diseases because of their exquisite specificity in binding to an antigen and ability to activate an immune response through effector functions like antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). The effector functions are triggered when an antibody Fc domain interacts with Fcγ receptors present on immune effector cells like natural killer cells and macrophages. However, traditional methods for characterizing these interactions are highly variable and labor-intensive.

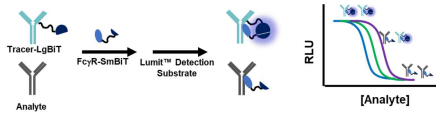
To meet the need for reliable, simple to use assays, we have developed a comprehensive suite of bioluminescent assays. The assays adhere to ICH guidelines and are designed to robustly and efficiently measure effector functions across the developmental workflow. The include FcγR binding immunoassays, cell-based reporter assays, and direct cell killing assays:

- Lumit® FcγR Binding Immunoassays
- Lumit® C1q Binding Assay
- Fc Effector Reporter Bioassays for ADCC and ADCP
- HiBiT Target Cell Killing Bioassays using MoA-qualified primary cells

Here we present a set of data on how these assays can be used to characterize anti-CD20 antibodies in the context of biosimilar antibody development. Utilizing these assays, our solutions enable researchers across the antibody development pipeline from early-stage discovery through commercial production and lot release.

2. Lumit® FcγR Binding Immunoassays: Concept, Format, and Workflow

Lumit® FcγR Binding Immunoassays are competition immunoassays based on NanoBiT® protein complementation technology.



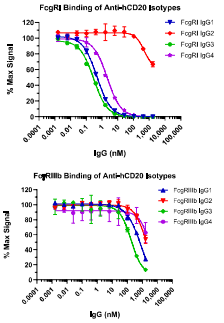
Kit Format for Lumit® FcγR Binding Immunoassays

- Control Antibody
- Tracer-LgBiT
- FcγR-SmBiT
- Lumit® Detection Substrate
- FcγR Assay Buffer

Simple add-and-read protocol

3. Lumit® FcγRI, FcγRIIb, and FcγRIIIb Binding Immunoassays

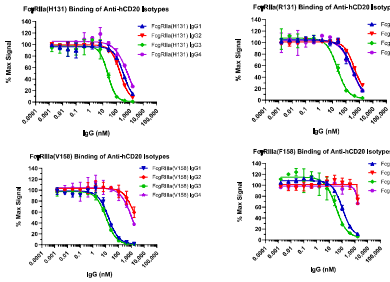
- FcγRI is the high-affinity receptor for the Fc region of IgG expressed on phagocytes and antigen-presenting cells, mediating IgG-dependent phagocytosis, and antibody-driven effector functions.
- FcγRIIb is the only inhibitory Fcγ receptor, expressed on B cells and myeloid cells, that binds IgG immune complexes to dampen immune cell activation.
- FcγRIIIb is a low-affinity, Fcγ receptor expressed on neutrophils that binds IgG immune complexes to facilitate their clearance and trigger neutrophil activation and degranulation.



Lumit® FcγR Binding Immunoassays are suitable for rank ordering different IgG isotypes based on their affinity to the different FcγRs, ideally suited for screening biosimilar binding kinetics.

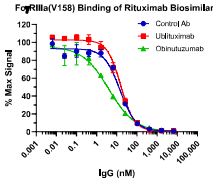
4. Lumit® FcγRIIa-H131 and -R131 and FcγRIIIa-V158 and -F158 Binding Immunoassays

- Two alleles of the gene coding for FcγRIIa generate two isoforms: H131 and R131. Antibody-Dependent Cellular Phagocytosis (ADCP) is triggered when IgG binds to FcγRIIa expressed on macrophages.
- Two alleles of the gene coding for FcγRIIIa generate two isoforms: V158 and F158. Antibody-dependent cellular cytotoxicity (ADCC) is triggered when antibodies bind to FcγRIIIa receptors on Natural Killer (NK) cells.



Lumit® FcγR Binding Immunoassays are suitable for rank ordering different IgG subclasses based on their affinity to the different FcγRIIa and FcγRIIIa allelic variants, factors for ADCP and ADCC function.

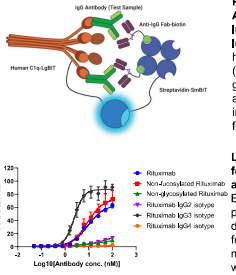
5. Lumit® Binding Immunoassays are Suitable for Screening Biosimilars



	IC50 (nM)	Max Signal (RLU)
Control Ab	14.21	1,458,000
Ublituximab	15.54	1,412,000
Obinutuzumab	3.971	1,492,000

Lumit® FcγR Binding Immunoassays can be used to distinguish differences in binding between two on-market anti-CD20 biosimilars.

6. Lumit® C1q Binding Assay accurately detects binding differences across antibody variants



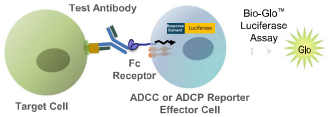
Principle of the Lumit® C1q Binding Assay : The assay is based on a split luciferase technology, half of the luciferase (LgBiT) is bound to C1q and half is bound to anti-IgG Fab tracer (SmBiT). A luminescent signal is generated only when the labelled C1q and an anti-IgG Fab tracer are brought into close vicinity by the test antibody, forming an active NanoBiT® luciferase

Lumit® C1q binding assay is suited for distinguishing antibody specificity and sensitivity. The Lumit® C1q Binding Assay was tested against a panel of Rituximab variants that included different IgG isotypes as well as fucoslyation and glycosylation modifications. The binding profiles align with published literature

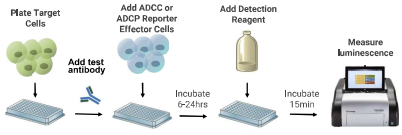
6. Fc Effector Reporter Bioassays: Concept, Format, and Workflow

Assay Principle and Workflow:

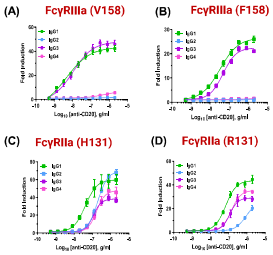
(A) Assay Principle: The Fc Effector Bioassays consist of two cell lines, target cells and the reporter cells. When co-cultured with an IgG antibody, the FcγR is activated and triggers promoter-driven luminescence that is detected using the Bio-Glo™ Luciferase Assay System.



(B) Assay Workflow: The Fc Effector Bioassays are designed for a simple add-mix-read format that standardizes the reagents to reduce variability over traditional methods, like primary cell-based assays that can result in high background signals.



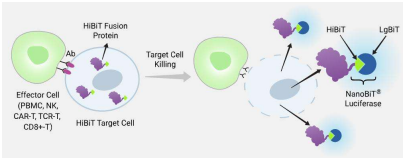
7. FcγR Reporter Assays Measure Antibody Potency



The **ADCC Reporter Bioassay** reflects the MOA and specificity of antibodies designed to bind and activate FcγRIIIa while demonstrating appropriate isotype specificity Single FcγRs are expressed in a reporter cell. **(A)** FcγRIIIa (V158) Bioassay **(B)** FcγRIIIa (F158) Bioassay

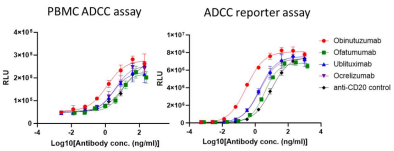
The **FcγRIIa ADCP Reporter Bioassay** reflects the MOA and specificity of antibodies designed to bind and activate FcγRIIa. The data above use anti-CD20 in the presence of Raji target cells **(C)** FcγRIIa (H131) ADCP Bioassay. **(D)** FcγRIIa (R131) ADCP Bioassay.

8. NanoBiT® Technology Enables Novel HiBiT Target Cell Killing Assays



- Incubation of effector cells and HiBiT-expressing target cells leads to tumor- associated antigen (TAA) dependent lysis of target cells and release of HiBiT into the medium.
- HiBiT binds to cell-impermeable LgBiT in the Bio-Glo-NB™ TCK detection reagent to form functional NanoBiT® luciferase and emit light.
- This technology enables rapid, MOA-based potency testing for biologics and cell therapy products.

9. HiBiT TCK Platform Measures Target Cell Killing for Biosimilar Bridging Studies



Assay	Measurement	Obinutuzumab	Ofatumumab	Ublituximab	Ocrelizumab	anti-CD20 Control
ADCC Reporter Bioassay	EC_{50} (ng/ml)	0.2887	3.675	1.842	1.888	9.335
PBMC ADCC Assay	EC_{50} (ng/ml)	1.28	7.250	2.829	3.831	15.22

HiBiT TCK Bioassays reflect the ADCC MoA of biologic drugs. The anti-CD20 biosimilars indicated were incubated with Human PBMC, ADCC-qualified effector cells or the ADCC Bioassay with serial titrations of the indicated antibody.

10. Conclusions

Comprehensive assay continuum from binding to function – An integrated suite of Lumit® FcγR binding, Fc Effector Reporter, and HiBiT Target Cell Killing (TCK) assays provides a coherent workflow that tracks an antibody's Fc effector activity from initial screening through lot release.

Rapid, scalable, and mechanism-aligned

- *Lumit® FcγR Binding Immunoassays* deliver subtype-specific binding and affinity ranking in < 60 min without immobilization artifacts, enabling high-throughput primary screens (96- to 384-well) or orthogonal confirmation to SPR/BLI.
- *Fc Effector Reporter Bioassays* employ thaw-and-use cells expressing individual human FcγRs; ICH-compliant precision, accuracy, and linearity for identifying binding changes that translate into functional potency shifts.
- *HiBiT TCK* couples NanoBiT luminescence from the target cell to authentic primary PBMC or $CD8^+$ T-cell activity, providing quantitative, no-wash cytotoxicity readouts that bridge binding and reporter potency data to physiologic cell-killing—ideal for bridging and comparability studies.

Cross-tier data concordance – Across multiple anti-CD20 variants, rank order observed in Lumit® IC_{50} values was preserved in reporter EC_{50} and TCK lysis, demonstrating predictive power for downstream cytotoxicity.

Time and risk reduction – Uniform plate-map architecture and assay quality cut assay transfer time from weeks to days and remove inter-assay reconciliation steps, supporting accelerated IND and BLA filings.

Regulatory-ready design – Assays are developed in accordance with ICH guidelines with system-suitability controls, defined acceptance criteria, and lot-specific CoAs, facilitating incorporation into comparability, stability, and release protocols.

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