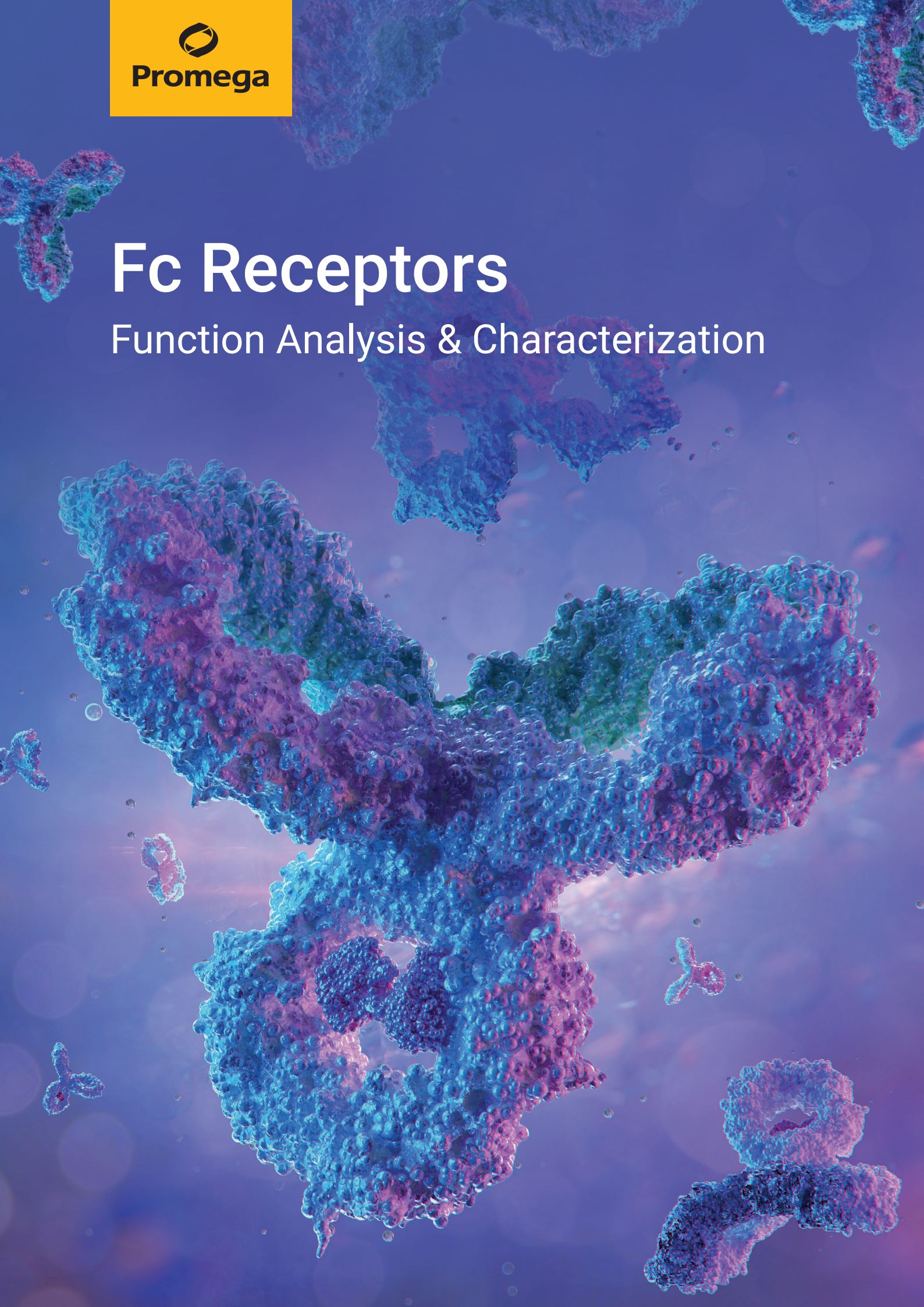
Promega

Fc Receptors

Function Analysis & Characterization



Fc Effector Activity and Binding Assays

Monoclonal antibody therapeutics have revolutionized the treatment of a wide range of cancer and autoimmune disorders. These immunomodulatory molecules act through a variety of mechanisms; but one of the most important is mediated through an antibody's interaction with Fc receptors. Upon binding antibodies associated with diseased target cells or pathogens, these Fc receptors mediate effector cell functions such as **antibody-dependent cellular cytotoxicity (ADCC)** and **antibody-dependent cellular phagocytosis (ADCP)**.

Promega has developed a broad suite of bioluminescent tools that enable biologic drug developers to assess their antibody therapeutic Fc effector function from lead generation through lot release:

- Measure **Fc receptor binding affinity** with **Lumit™ Immunoassays** in a simple, scalable, and rapid assay format.
- Quantify **ADCC or ADCP activity** in lot release potency settings with **cell-based reporter assays** reflecting the **mechanism of action (MOA)** of your therapeutics.
- Perform **bridging studies** in a physiologically relevant **primary cell model**. The **HiBiT target cell killing assays** use PBMCs qualified for ADCC activity to save time and provide highly consistent results.

Fc RECEPTOR BINDING ASSAYS

Biochemical binding immunoassays

- Analyze antibody-Fc-receptor interactions
- Determine the serum half-life of monoclonal antibodies and effector mechanisms
- High-throughput screening of Fc-engineered antibody libraries

Fc EFFECTOR POTENCY ASSAYS

Cell-based assays using engineered ADCC or ADCP reporter effector cells

- MOA-based assays to measure Fcγ receptor function
- Use for lot release and stability studies of monoclonal antibodies
- Discriminate levels of glycosylation and fucosylation of monoclonal antibodies
- Reduce variation compared to traditional primary cell assays

TARGET CELL KILLING ASSAYS

Primary cell assays combined with engineered HiBiT-expressing target cells

- Measure target cell-specific killing of ADCC-qualified effector cells
- Human cell model with high physiological relevance
- Enable bridging studies from drug discovery and characterization to lot release

PHYSIOLOGICAL RELEVANCE

FROM LEAD GENERATION TO LOT RELEASE

Benefit from powerful bioluminescence tools ranging from biochemical *in vitro* studies to physiologically relevant primary cell assays to analyze Fc effector function throughout your biologic drug development process.

Fc Receptor Binding Assays

Measure the interaction between a therapeutic monoclonal antibody (mAb) and the human neonatal Fc receptor (FcRn) or Fcγ receptors (FcγR) with **Lumit™ Fc Receptor Binding Immunoassays**. The assays require no immobilization or washing steps, making them completely homogenous.

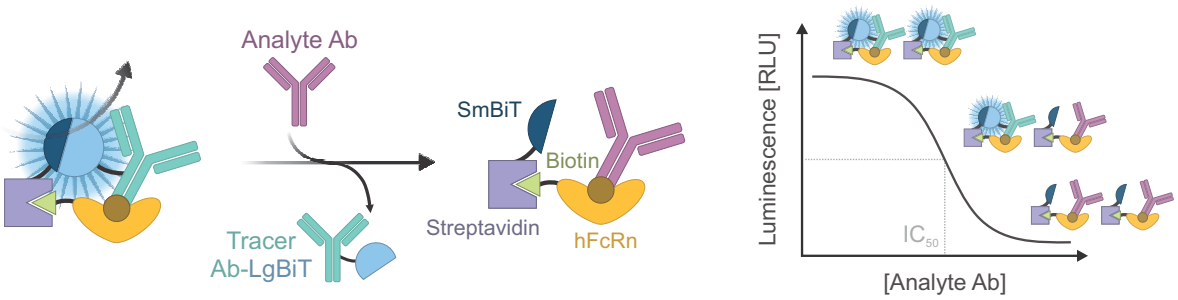
Lumit™ Immunoassays are based on the versatile NanoBiT® complementation reporter composed of two complementary subunits, i.e., LgBiT and SmBiT. The latter was engineered to show minimal affinity (K_d = 190 µM) for the LgBiT counterpart, which makes NanoBiT® ideally suited for interaction analyses. The interaction of two proteins, labelled with either LgBiT or SmBiT, reconstitutes the functional NanoBiT® luciferase that generates a stable and bright luminescent signal in the presence of its substrate.

The Fc binding assays utilize a LgBiT-labeled hIgG1 (Tracer Ab-LgBiT) and a biotinylated FcR bound to SmBiT-labeled streptavidin (hFcR-Biotin-Streptavidin-SmBiT). In the absence of an analyte antibody (Analyte Ab), Tracer-binding to labeled hFcR results in a maximum luminescent signal. The binding of analyte antibodies is evident from a concentration-dependent signal. The signal decreases with competitive tracer displacement by analyte antibodies.

Lumit™ Fc Receptor Binding Immunoassays are available for human FcRn and three different human FcγRs as well as important polymorphic variants:

- FcRn
- FcγRI
- FcγRIIa (H131)
- FcγRIIa (R131)
- FcγRIIIa (V158)
- FcγRIIIa (F158)

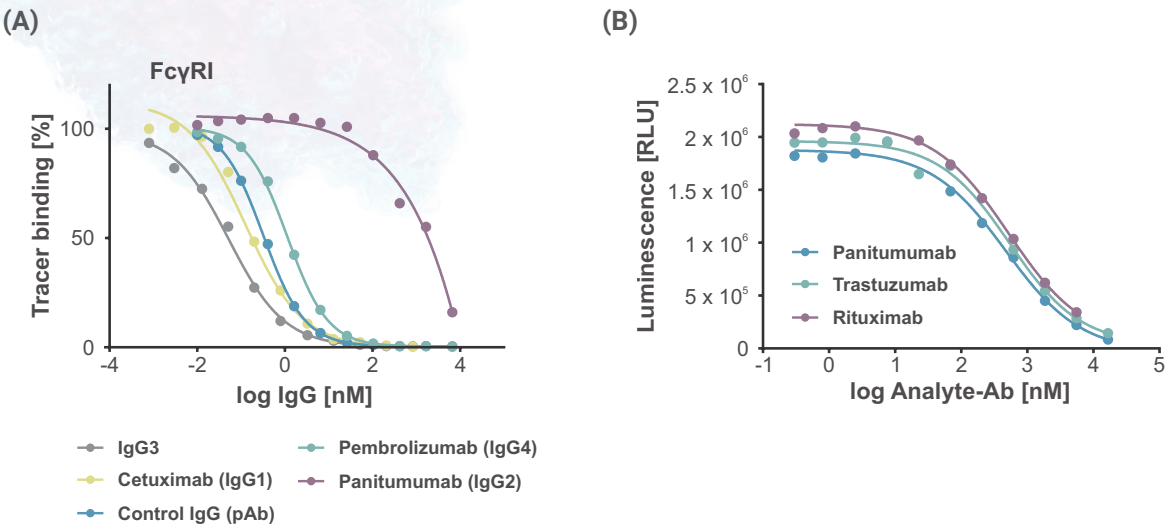
Assay Principle



Features & Benefits

- Parallel & automated Fc affinity screening of mAbs
- No immobilization artifacts
- Sensitive bioluminescent readout
- Cell-free, solution-based without washing or transfer
- Results in less than 70 minutes
- Scalable to 384-well format

Representative Data



(A) Lumit™ FcγRI Binding Immunoassay. FcγRI binds to IgG in a subclass-specific manner in the Lumit™ Immunoassay with IC_{50} values reflective of relative IgG affinity (IgG3 > IgG1 > IgG4 >>> IgG2). **(B) Lumit™ FcRn Binding Immunoassay.** A panel of therapeutic antibodies was tested for their affinity to FcRn.

References

» Nath, N. *et al.* (2022) A homogeneous bioluminescent immunoassay for parallel characterization of binding between a panel of antibodies and a family of Fc receptors. Sci Rep 12, 12185.

» Nath, N. *et al.* (2021) Deciphering the interaction between neonatal Fc receptor and antibodies using a homogeneous bioluminescent immunoassay. J Immunol. 207(4), 1211–1221.

» Tian, Z. *et al.* (2021). Harnessing the power of antibodies to fight bone metastasis. Sci Adv. 7(26), eabf205

Discover the versatile possibilities of Lumit™ technology for the detection of proteins and protein interactions in microtiter plates: **www.promega.com/Lumit-brochure**

Fc Effector Potency Assays

Measure the potency and stability of therapeutic antibodies or other biologics that bind or activate Fc receptors with cell-based **ADCC and ADCP Reporter Assays**.

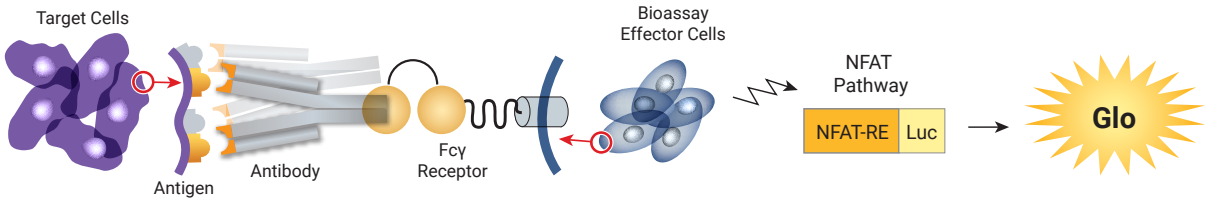
The assays consist of Jurkat or THP-1 effector cells stably expressing the relevant Fcγ receptor variant and a firefly luciferase reporter gene regulated by an NFAT response element (NFAT-RE). Following engagement with the Fc region of a relevant antibody bound to a target cell, effector cells expressing the relevant Fcγ receptor transduce intracellular signals resulting in NFAT-mediated luciferase activity that can be easily quantified.

The workflow is simple, compatible with 96-well and 384-well plate formats, and, unlike traditional primary cell-based assays, provides a quantitative measure of ADCC and ADCP with low variability and high accuracy.

Fc Effector Potency Assays have been developed to quantify antibody-mediated signaling through the following receptors:

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

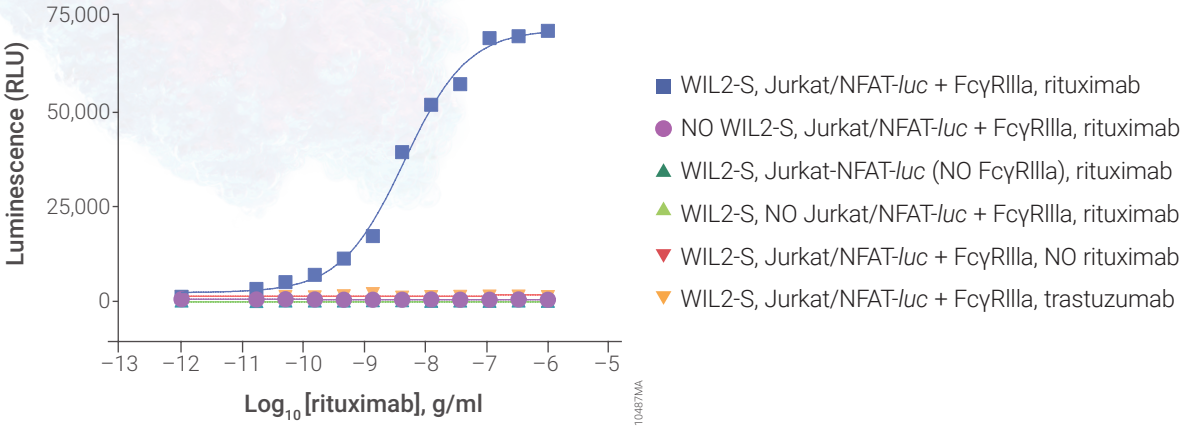
Assay Principle



Features & Benefits

- Biologically relevant measurement of antibody MOA
- Prequalified according to ICH guidelines
- Correlates with primary cell assays
- Low variability compared to traditional primary cell assays
- Thaw-and-use cell format
- Scalable to 348-well format

Representative Data



Serial dilutions of Rituximab (anti-CD20), Trastuzumab (anti-Her2) or assay medium control (no antibody) were incubated for 6 hrs at 37°C with engineered Jurkat Effector Cells (ADCC Bioassay Effector Cells) with or without ADCC Bioassay Target Cells (WIL2-S), as indicated.

References

» Zhang, X., *et al.* (2019) A recombinant human IgG1 Fc multimer designed to mimic the active fraction of IVIG in autoimmunity. JCI InSight 4, e121905.

» Hu, Z., *et al.* (2018) Targeting tissue factor for immunotherapy of triple-negative breast cancer using a second-generation ICON. Cancer Immunol. Res. 6, 671–684.3.

» Kommineni, V., *et al.* (2019) In vivo glycan engineering via the mannosidase I inhibitor (kifunensine) improves efficacy of Rituximab manufactured in Nicotiana benthamiana plants. Int. J. Mol. Sci. 20, 194.

» Kauder, S.E., *et al.* (2018) ALX148 blocks CD47 and enhances innate and adaptive antitumor immunity with a favorable safety profile. PLoS ONE 13, e0201832.

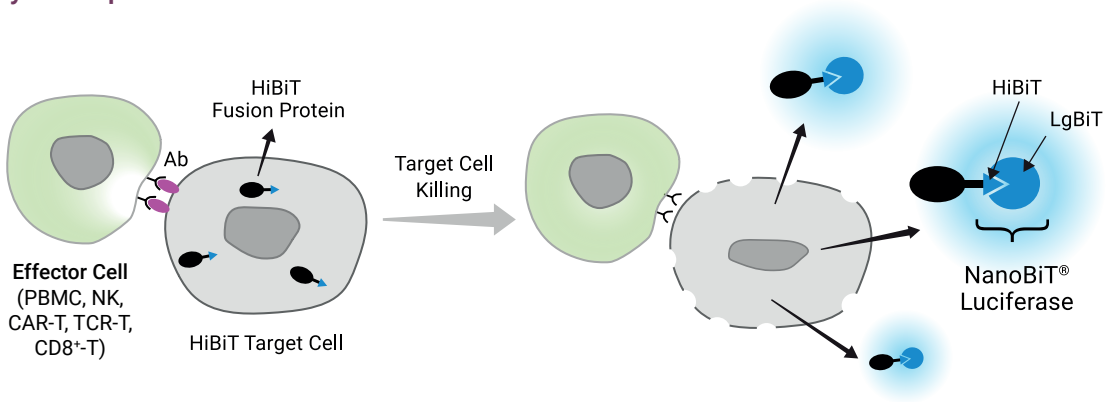
For a detailed overview of Fc Effector Potency Assays and other MOA-reflecting bioassay for your biologics, download our brochure: **www.promega.com/BioassaysForBiologics**

Target Cell Killing Assays

Measure target cell killing induced by a variety of biologic drugs, including monoclonal antibodies (mAbs), bispecific T-cell engager (BiTE) antibodies, T cell receptor engineered T cells (TCR-T), or chimeric antigen receptor T cells (CAR-T) with the **HiBiT target cell killing (TCK) platform**.

The TCK assays are based on engineered target cell lines stably expressing a HiBiT fusion protein. HiBiT is an 11-amino acid peptide tag with high binding affinity in the picomolar range for the complementary LgBiT subunit of the NanoBiT® luciferase. Upon lysis of the target cell through incubation with effector cells and/or an antibody, HiBiT is released into the cell culture supernatant, where it can be easily detected through the addition of LgBiT. The reconstituted NanoBiT® luciferase emits a stable and bright luminescent signal after reagent addition. The signal correlates with the number of dead cells.

Assay Principle



Target Cells

HiBiT target cells can be used in combination with different primary effector cells, e.g., peripheral blood mononuclear cells (PBMC), natural killer cells (NK), CD8+ T cells (CD8+-T), CAR-T, or TCR-T cells.

HiBiT target cells are available for most popular immunotherapeutic targets (e.g., CD19, CD20, BCMA, and more) as cryopreserved thaw-and-use cells or as cell propagation models. Since we are constantly working on the development of new cell lines, we recommend contacting us for an updated list of all available HiBiT target cells.

YOU NEED A SPECIFIC TARGET CELL LINE?

Promega's **Biologics Assay Development and Services** offer custom cell line generation, assay development, and more: **www.promega.com/CustomBiologics**

Primary Effector Cells (ADCC-qualified)

Promega offers, in partnership with BioIVT, QC-tested, thaw-and-use primary effector cells to measure ADCC of therapeutic antibodies.

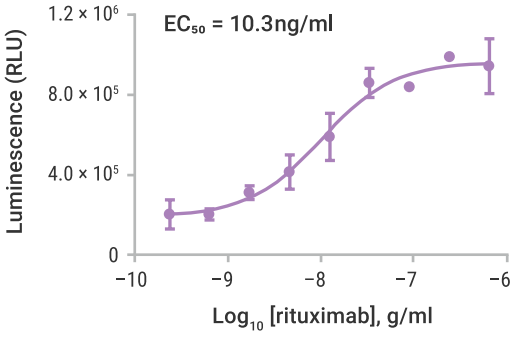
Primary cells are available as standalone vials or as part of a bioassay kit including a choice of HaloTag®-HiBiT target cells, FBS, cell culture medium, and Nano-Glo® HiBiT Extracellular Detection System.

The combination of qualified primary effector cells and HiBiT target cells overcomes the limitations of labor-intensive and highly variable traditional primary cell assays with freshly isolated PBMCs or NK cells from donor blood, and offers simple, specific, and sensitive determination of TCK.

Features & Benefits

- Screening & potency testing for ADCC
- Physiologically relevant human cell model
- High specificity in co-cultures
- Endpoint & kinetic formats
- Choice of HiBiT target cells for popular immunotherapy targets
- No blood isolation and processing: High-quality primary effector cells, as thaw-and-use cells for maximum convenience and minimal assay variability

Representative Data



PBMC ADCC Bioassay: PBMC-mediated killing of Raji (HiBiT) Target Cells by anticancer therapeutic mAb, rituximab.

Reference

» Garvin, D. *et al.* (2021). Determining ADCC activity of antibody-based therapeutic molecules using two bioluminescent reporter-based bioassays. Current Protocols 1, e296. doi: 10.1002/cpz1.296

Ordering Information

Fc Receptor Binding Assays

Product	Size	Cat.#
FcRn Binding Immunoassay	100 assays 1000 assays	W1151 W1152
FcγRI Binding Immunoassay	100 assays	CS3041A01
FcγRIIA (H131) Binding Immunoassay	100 assays	CS3041A02
FcγRIIA (R131) Binding Immunoassay	100 assays	CS3041A03
FcγRIIIA (V158) Binding Immunoassay	100 assays	CS3041A04
FcγRIIIA (F158) Binding Immunoassay	100 assays	CS3041A05

Fc Effector Potency Assays

Product	Size	Cat.#
Human ADCC Reporter Bioassays		
FcγRIIIa (V158) ADCC Reporter Bioassay (**Raji** Target Cells)	Complete Kit[1] Core Kit[2] 1x or 5x Target Kit[3] Cell Propagation Model[4]	G7015 G7010, G7018 G7016 G7102
CD38-KO ADCC Reporter Bioassay	Core Kit[2] 1x or 5x Propagation Model[4]	CS313304, CS313306, CS313302
FcγRIIIa (F158) ADCC Reporter Bioassay	Core Kit[2] 1x or 5x Cell Propagation Model[4]	G9790, G9798 G9302
Target Cells for ADCC Reporter Bioassays		
Membrane TNFα Target Cells	Thaw-and-Use Cells[5] (1x or 5x) Cell Propagation Model[4]	J3331, J3335 J3322
Membrane VEGF Target Cells	Thaw-and-Use Cells[5] (1x or 5x) Cell Propagation Model[4]	J3351, J3355 J3342
Membrane RANKL Target Cells	Thaw-and-Use Cells[5] (1x or 5x) Cell Propagation Model[4]	J3381, J3385 J3362
Mouse ADCC Reporter Bioassays		
mFcγRIV ADCC Reporter Bioassay	Complete Kit[1] Core Kit[2] 1x or 5x Cell Propagation Model[4]	M1201 M1211, M1215 M1212
mFcγRIII ADCC Reporter Bioassay	Core Kit[2] Cell Propagation Model[4]	CS1779B08, CS1779B06

Human ADCP Reporter Bioassays		
FcγRIIa-H ADCP Reporter Bioassay	Complete Kit[1] Core Kit[2] 1x or 5x Cell Propagation Model[4]	G9901 G9991, G9995 G9871
FcγRIIa-R ADCP Reporter Bioassay	Core Kit[2] 1x Cell Propagation Model[4]	CS1781B08 CS1781B06
FcγRI ADCP Reporter Bioassay	Core Kit[2] 1x or 5x Cell Propagation Model[4] Cell Bank[6]	GA1341, GA1345 GA1323 GA1330
THP-1 ADCP Reporter Bioassay	Core Kit[2] 1x or 5x Cell Propagation Model[4]	CS314904, CS314906 CS314902
Other FcγR Reporter Bioassays		
FcγRIIb Reporter Bioassay	Core Kit[2] 1x or 5x Cell Propagation Model[4]	CS1781E02, CS1781E04 CS1781E07
Control Ab, Anti-CD20	1x 5 µg	GA1130

Target Cell Killing Assays

Product	Size	Cat.#
Human PBMC, ADCC-qualified	Thaw-and-Use Cells[5] (1x or 5x)	CS3055A15, CS3055A18
PBMC ADCC Bioassay Kit (**Raji**)	1x or 5x	CS3055A14, CS3055A17
PBMC ADCC Bioassay Kit (**Ramos**)	1x or 5x	CS3055A20, CS3055A22
PBMC ADCC Bioassay Kit (**SK-BR-3**)	1x or 5x	CS3055A36, CS3055A38
PBMC ADCC Bioassay Kit (**H929**)	1x or 5x	CS3055A28, CS3055A30
PBMC ADCC Bioassay Kit (**A549**)	1x or 5x	CS3055A24, CS3055A26
PBMC ADCC Bioassay Kit (**SKOV3**)	1x or 5x	CS3000A43, CS3000A44
Target Cells		
HaloTag®-HiBiT Target Cells *(e.g., Raji, Ramos, A539, SK-BR-3, H929, U937, OVCAR3, SKOV3 T2, etc.)* *Each target cell can be paired with PBMCs.*	Thaw-and-Use Cells[5] (1x or 5x) Cell Propagation Model[4]	*Please enquire for complete list*

1 Complete Kit: Thaw-and-use effector cells, target cells, control antibody, cell plating and assay reagents
2 Core Kits: Thaw-and-use effector cells, cell plating and assay reagents
3 Target Kits: Thaw-and-use target cells, control antibody
4 Propagation Model: 2 vials of cryopreserved cells that can be thawed, propagated, and banked for long-term use
5 Thaw-and-Use Cells: 1 or 5 vials of cryopreserved cells for single usage (no propagation)
6 Cell Bank: 50 vials of cryopreserved cell bank that can be thawed and propagated for functional testing

Note: CS product numbers indicate Early Access (EA) Products and cannot be found in the catalog or online.
If you need more information about an EA product, please contact us: **TailoredSolutions@promega.com**

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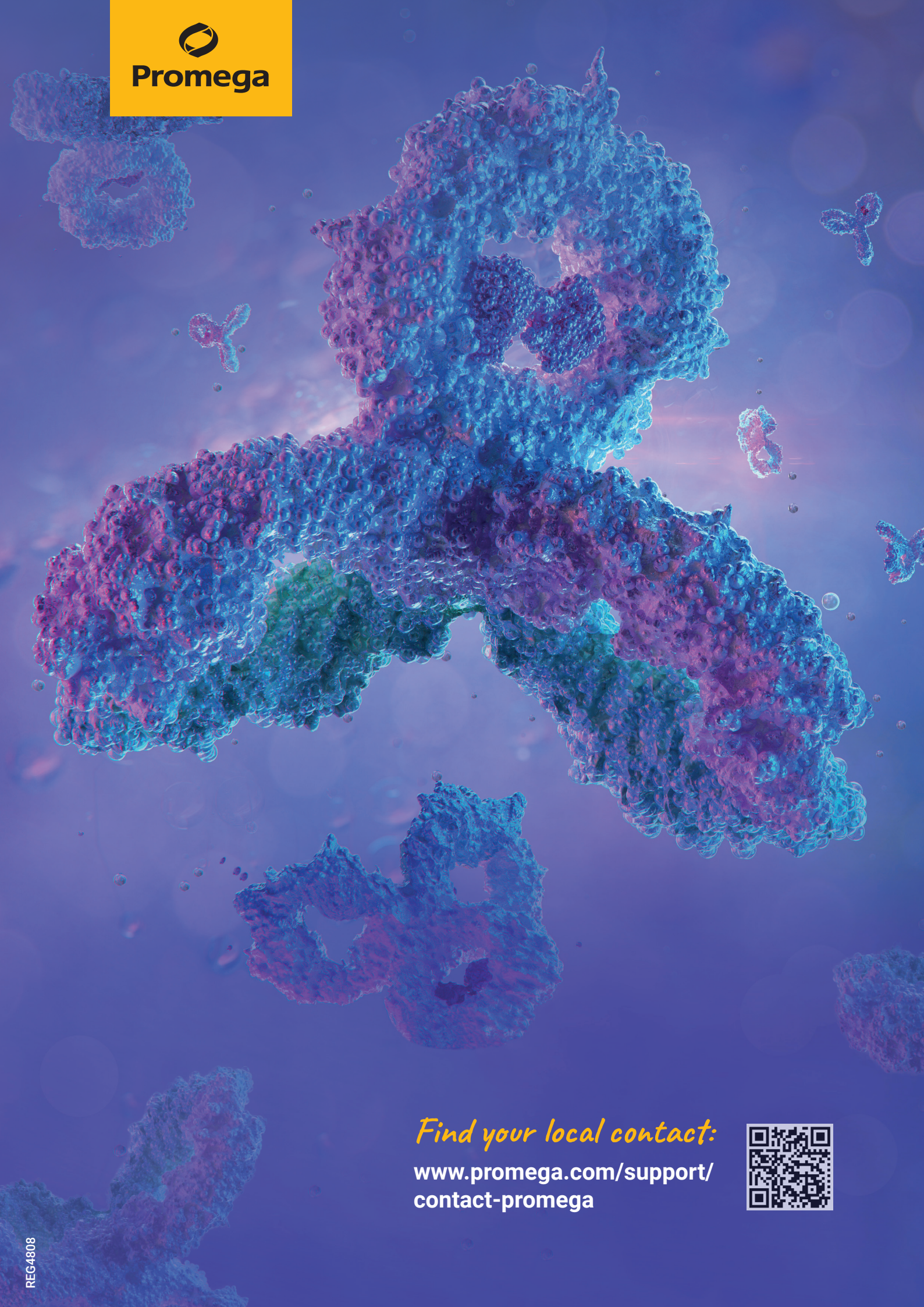
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