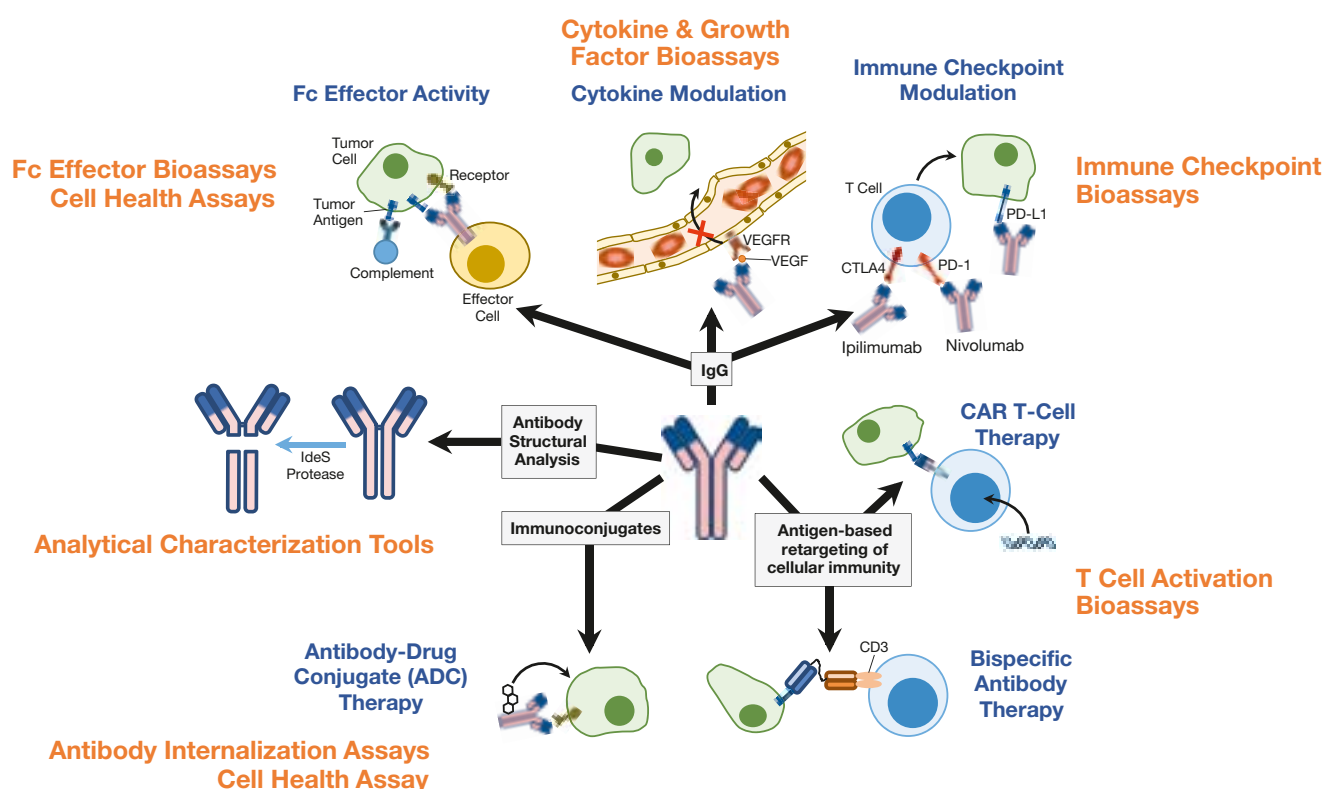


# Promega Biologics Portfolio

## Functional Analysis of Antibody-Based Biologics



Adapted from Weiner, G.J. (2015) *Nat. Rev. Cancer* **15**, 361

Antibody-based therapeutic drugs can be broadly classified into six categories depending on their mechanism of action (MOA), as indicated in the schematic. Many biologic drugs function via a single mechanism; however, newer drug candidates are being designed that function through multiple mechanisms. The use of MOA-based bioassays to measure the activity of drug candidates is critical throughout the drug development workflow.

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# Signaling Pathway Based Bioassays for Biologics

## Promega Biologics Benefits

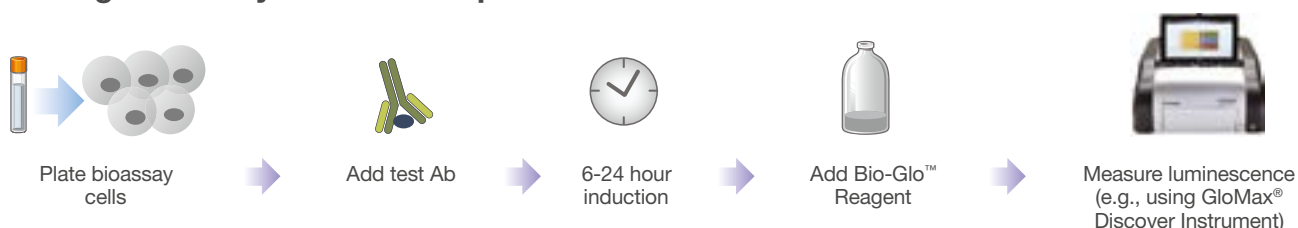
- ✓ Mechanism of action (MOA)-based bioassays
- ✓ Easy to implement “thaw-and-use” vials enable consistent, reproducible results
- ✓ Accurate, precise and reproducible
- ✓ Ideal for QC and lot release of biologic drugs
- ✓ Useful for potency and stability determination
- ✓ Amenable to high-throughput formats
- ✓ Prequalified according to ICH guidelines
- ✓ Sensitive and homogeneous detection reagents to quantify reporter gene expression

## Overview

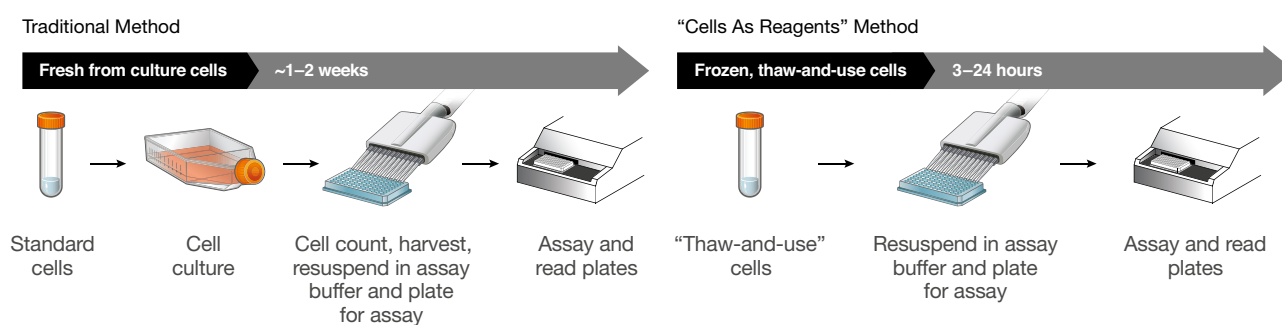
In contrast to small molecule drugs that are chemically synthesized and have a known structure, biologics are large molecules with complex, heterogeneous structures that are often unstable and sensitive to external conditions. Due to their high degree of complexity, the development of biologic drugs requires a comprehensive set of quantitative, accurate and precise bioanalytical tools. Promega offers an extensive toolbox of reporter bioassays to characterize and develop novel monoclonal antibody (mAb)-based therapeutics. These assays are useful for interrogating a range of biological functions, including Fc effector activity, immune checkpoint modulation, T cell activation, and cytokine and growth factor signaling. We maintain a dynamic pipeline of bioassays and can partner with drug developers in the early stages of development to ensure that our bioassays meet your needs.

All bioluminescent cell-based bioassays are available as “thaw-and-use” format vials or as cell lines for propagation in continuous culture. Some of the described assays are available as catalog items (Cat. no.) whereas custom assay materials have not been officially launched yet. Custom materials (indicated by CS numbers) are developed by a special Promega Custom Assay Services (CAS) team. CAS products are functionally tested but are not yet manufactured under ISO guidelines and include no usual warranties. Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

### Promega bioassays follow a simple “add-mix-read” format.



**Promega bioassays offer a rapid and simple workflow compared to traditional methods.**



### **Convenient “thaw-and-use” cells as single use format**

All bioassay cell lines are available in “thaw-and-use” format. Cells provided as “thaw-and-use” have been optimized for single use in the assays. This greatly minimizes assay variability and simplifies experiment planning and logistics. “Thaw-and-use” cells are not designed for continuous cell culture propagation and therefore can only be utilized for one experiment. They serve as a tool for assay development and characterization of biologics in an R&D environment for drug discovery, development and monitoring of biologics, vaccines and product release.

### **Cell propagation model (CPM) for continuous propagation in cell culture**

As an alternative to “thaw-and-use” cells, cell lines for continuous propagation in culture allow researchers to create and manage their own master and working cell banks for future use. CPM formats contain cryopreserved cells can be thawed and propagated for long-term use. It is highly recommended to first start with the “thaw-and-use” cells in order to optimize the assay conditions.

### **Bio-Glo™ Luciferase Assay Systems for a simple and robust detection**

Promega’s bioluminescent bioassays have been developed by using luciferase reporters expressed under the control of an appropriate response element/promoter. This allows to study activation or inhibition of the pathway relevant to the tested biologic and corresponding target molecules. Detection of the reporter signal is achieved by measuring luminescence using Promega’s highly sensitive, robust and homogeneous Bio-Glo™ Luciferase Assay Systems. The reagents are more stable and have an improved tolerance to sample components than standard luciferase assays. The bioassays are either based on the luciferase reaction of the firefly luciferase (Bio-Glo™ Luciferase Assay System) or of the NanoLuc® luciferase (Bio-Glo-NL™ Luciferase Assay System). Bioassays that are based on the NanoLuc® luciferase are highlighted in the ordering information of each section. Bio-Glo™ Assay reagents are functionally tested for performance and are intended for use in Promega’s Reporter Bioassays.

# Fc Effector Bioassays

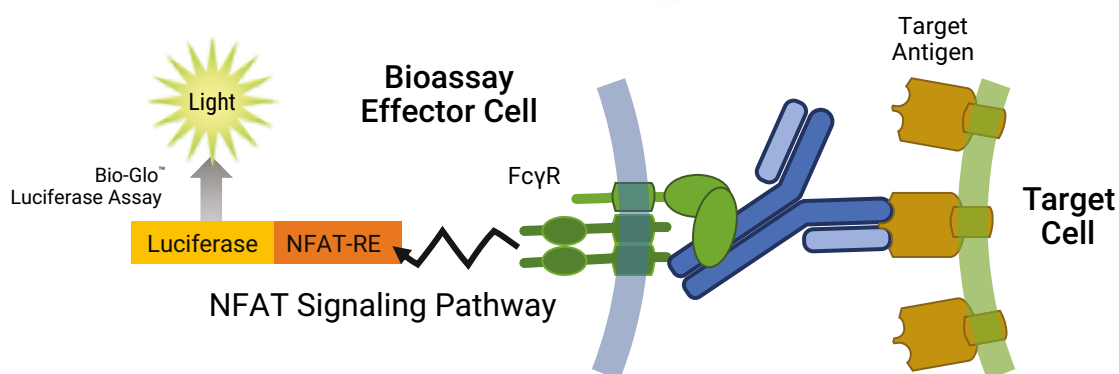
## Benefits

- ✓ Cell-based reporter bioassay platform to measure ADCC and ADCP mediated through Fc $\gamma$  receptors
- ✓ Cells express specific Fc $\gamma$  receptors and a NFAT response element, which drive luciferase-based reporters
- ✓ No reliance on inconsistent primary peripheral blood mononuclear cells (PBMCs)
- ✓ Scalable measurement of biologics potency and stability
- ✓ Currently used in lot release of multiple biologic drugs
- ✓ Correlation with primary cell-based ADCC assays
- ✓ Discriminate levels of glycosylation and fucosylation of antibodies

## Overview

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important mechanism of action (MOA) of antibodies that target virus-infected or diseased (e.g. tumor) cells for destruction by components of the cell-mediated immune system. Fc receptor-mediated effects contribute to the efficacy and safety of therapeutic antibodies to tumor necrosis factor (TNF). Human Fc $\gamma$ R1II is the predominant receptor involved in ADCC and exists as a high-affinity (Fc $\gamma$ R1IIa-V158) or low-affinity (Fc $\gamma$ R1IIa-F158) variant, depending on the amino acid at position 158. Although other Fc $\gamma$  receptors contribute, Fc $\gamma$ R1IIa is believed to be the predominant Fc $\gamma$  receptor involved in antibody-dependent cell-mediated phagocytosis (ADCP).

The human ADCC and ADCP and mouse ADCC Reporter Bioassays are biologically relevant, MOA-based assays that can be used to measure the potency and stability of antibodies and other biologics that specifically bind and activate Fc $\gamma$  receptors. The assays consist of Jurkat cells stably expressing the relevant Fc $\gamma$  receptor variant and a luciferase gene regulated by the NFAT (Nuclear Factor of Activated T cells) response element. The bioassays overcome the limitations of more labor-intensive and highly variable primary cell assays. 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 assay principle.** ADCC/ADCP Bioassay Effector Cells consist of Jurkat cells engineered to express human FcγR and a luciferase reporter driven by an NFAT response element (NFAT-RE). In the presence of antibody and target cells expressing the relevant antigen, the Effector Cells will transduce intracellular signals, resulting in NFAT-mediated luciferase activity that can be easily quantified.

Fc Effector Function Reporter Bioassays have been developed to quantify antibody-mediated signaling through the following receptors:

- **Human FcγRIIIa (V158 and F158 variants)**
- **Human FcγRIIIa (H131 and R131 variants)**
- **Human FcγRI**
- **Human FcγRIIb**
- **Mouse FcγRIV**
- **Mouse 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 the Fc Effector Function Bioassays exhibit excellent specificity, accuracy, precision and linearity enabling their use in antibody screening, characterization, stability, potency determination and lot release.

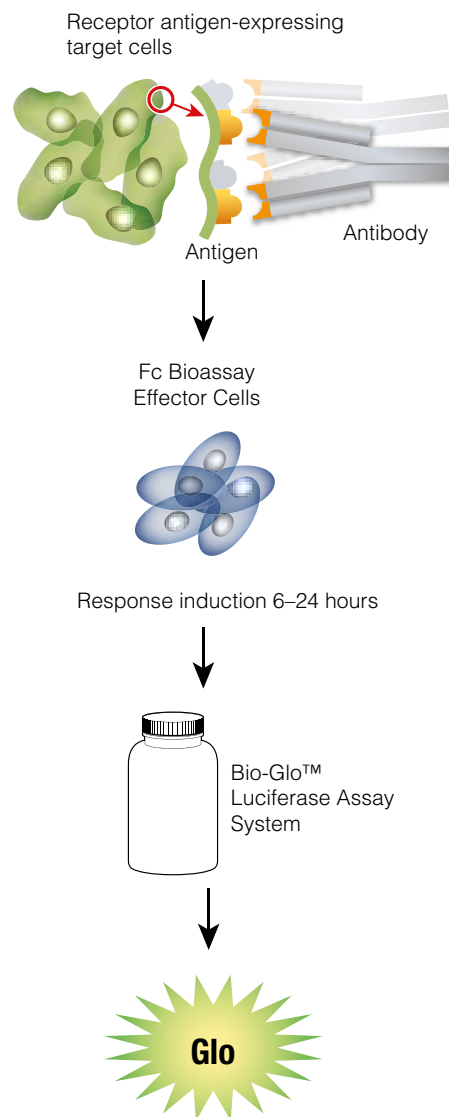
#### Recent Citations

1. 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.
2. 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.
4. 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.

## Fc Reporter Bioassay concept

Brief pre-incubation of antigen-presenting target cells with a therapeutic antibody is followed by addition of the genetically engineered Jurkat Effector Cells. Subsequent activation of the Fc receptor signaling pathway results in increased reporter gene (luciferase) expression, measured by simple addition of Bio-Glo™ detection reagent. Reporter gene measurements correlate with readouts in classic assays. By eliminating primary cells, assay variability is significantly reduced whilst retaining the ability to discriminate antibodies with varying degrees of Fc effector activity. Target cells (adherent/suspension) expressing the relevant antigens can be provided by the user to match the therapeutic antibody under test, although kit formats with a control target cell/antibody included are available for ongoing assay Quality Control (QC).

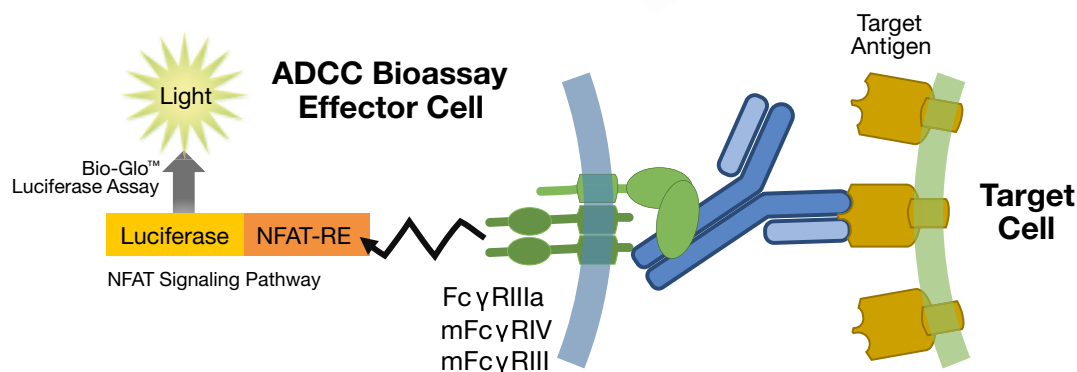
## Schematic protocol for the Fc Reporter Bioassay



By using engineered Effector Cells instead of primary cells, the assay reproducibility is greatly increased and the variability is significantly reduced while retaining the ability to discriminate antibodies with varying degrees of Fc effector function.



# Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Assays



**Fcγ effector assay principle:** The ADCC bioassays utilize genetically engineered stable Jurkat Effector Cells to express a specific human or mouse/murine Fc receptor and an NFAT response element that drives expression of luciferase. Following engagement with the Fc domain of a relevant antibody bound to target cells, signaling through the specific Fc receptor induces luciferase activity that is easily detected and quantified.

Traditional MOA-based ADCC assays are challenging, from isolating specific populations of cells from blood to maintaining well-controlled assay conditions. This is time consuming and can lead to highly variable results that are difficult to replicate.

ADCC Reporter Bioassays eliminate variability by providing frozen, “thaw-and-use” Effector Cells and quality-controlled reagents. In a reporter-based ADCC Bioassay the measured signal comes from the genetically engineered Effector Cells. It is a stable Jurkat cell line in which a luminescent reporter readout indicates activation of the ADCC signaling pathway due to activation of the NFAT response element.

**Read the paper  
about the design  
of this bioassay.**

Cheng, Z.J., et al. (2014) Development of a robust reporter-based ADCC assay with frozen, “thaw-and-use” cells to measure Fc effector function of therapeutic antibodies. *J. Immunol. Methods* **414**, 69–81.

## Recent Citations

1. Amanat, F., et al. (2019) Cross-reactive antibodies binding to H4 hemagglutinin protect against a lethal H4N6 influenza virus challenge in the mouse model. *Emerg. Microb. Inf.* **8**, 155–168.
2. Kosik, I., et al. (2019) Neuraminidase inhibition contributes to influenza A virus neutralization by anti-hemagglutinin stem antibodies. *J. Exp. Med.* **216**, 304–316.
3. Theunissen, J.-W., et al. (2018) Treating tissue factor-positive cancers with antibody-drug conjugates that do not affect blood clotting. *Mol. Cancer Therap.* **17**, 2412–26.

## Ideal Bioassay

The ADCC Reporter Bioassay has performance characteristics suitable for many applications across antibody drug discovery, development and manufacture. It is stability-indicating and has the precision and accuracy suitable for a lot-release bioassay. Additionally, the assay can be used to quantify effects of glycosylation differences on Fc effector function of antibodies in ADCC MOA and provides antibody activity ranking equivalent to classic cytotoxicity-based (LDH release) ADCC assays. Assay optimization and assay performance have been extensively tested using the FDA-approved antibodies Rituximab and Trastuzumab.

## F and V variants

ADCC is a Fc effector function involving binding of antigen-bound antibody Fc domains with the Fc $\gamma$ R11a receptor on immune system “killer” cells. Polymorphism in the Fc $\gamma$ R11a receptor at amino acid 158 results in both high affinity (V158) and low affinity (F158) variants Fc $\gamma$ R11a genotypes (e.g. VV, FV, FF) of individual patients and are correlated with clinical efficacy of some therapeutic antibody drugs. ADCC Reporter Bioassays for both high (V158) and low affinity (F158) Fc $\gamma$ R11a variants allow quantitative measurement of the potency of therapeutic antibodies in ADCC and evaluate the impact of Fc $\gamma$ R11a polymorphism in drug discovery and development.

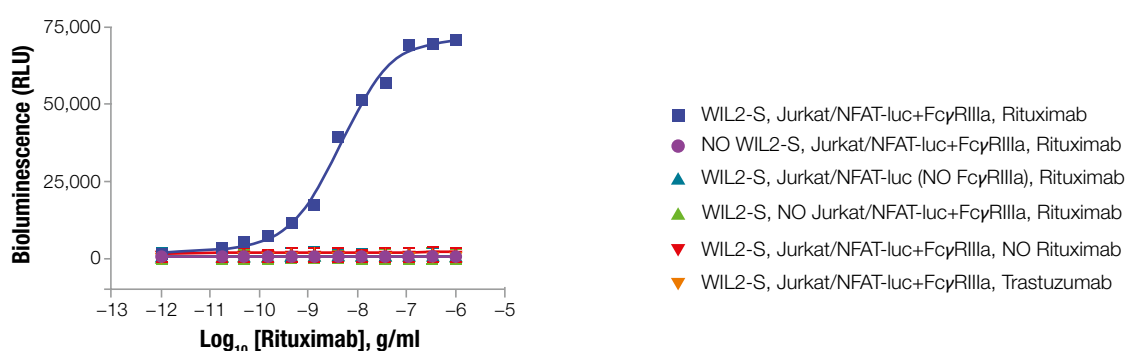
| Fc $\gamma$ R11a                         |  |
|--|--|
| 158 F/V or F/F                           | 158 V/V                                  |
| >85% population                          | ~10–15% population                       |
| Less efficient antibody binding and ADCC | More efficient antibody binding and ADCC |

## Bioassay Kit Formats

- Core Kits: include ADCC Bioassay Effector Cells, Medium, Low-IgG Serum, Bio-Glo™ Luciferase Reagent  
Recommended for routine use with customer-defined antibody and target cells.
- Complete Kits: include all Core Kit components, Target Cells, control Ab  
Recommended for use as a starter kit.

## Specificity of the ADCC Reporter Bioassay

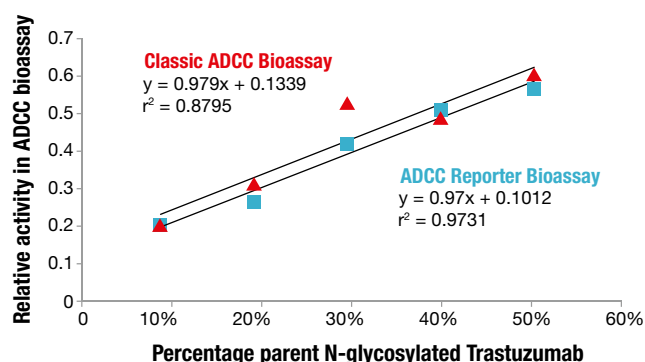
The ADCC Reporter Bioassay exhibits the distinct specificity desired for a bioassay. The reporter gene luminescent response is only present when target cells with the correct surface antigen, the correct specific antibody and effector cells expressing FcγRIIIa are present. If any of these is missing, no response is observed.



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.

## Correlation with classic ADCC data

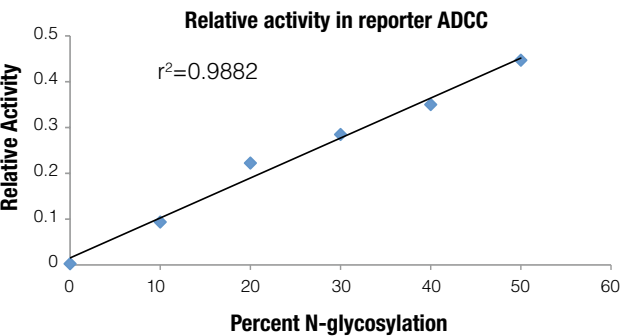
The ADCC Reporter Bioassay provides antibody activity ranking equivalent to classic LDH release ADCC Bioassay.



Correlation of relative ADCC activity with fraction of Trastuzumab N-glycosylation. Trastuzumab was N-deglycosylated using PNGase F, blended with fully N-glycosylated parent preparations to create test samples representing different % N-glycosylation (indicated on the X-axis) and assayed using either the ADCC Reporter Bioassay or a lytic LDH release ADCC bioassay in which PBMCs were used as Effector Cells. Target cells were SK-BR-3.

### Discriminate levels of glycosylation and fucosylation

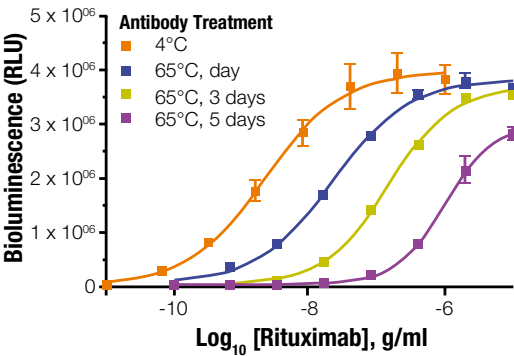
Determination of the effect of antibody glycosylation on Fc effector activity requires a robust assay to reliably detect changes in antibody potency associated with a slight change in glycosylation level. Data shown in the graph highlight that activity changes associated with small (5–10 %) changes in glycosylation level are easily detectable with the ADCC Reporter Bioassay.



**Antibody glycosylation: relative activity in ADCC Reporter Bioassay.** Rituximab-blended samples containing mixes of fully deglycosylated and fully glycosylated antibodies were assayed as serial dilutions against serial dilutions of a 100 % reference sample of fully N-glycosylated Rituximab using the ADCC Reporter Bioassay.

### Stability indicating

ADCC Reporter Bioassay is stability indicating and has been tested in this respect with the FDA-approved antibodies Rituximab and Trastuzumab.



|                  | 4°C                     | 65°C, day               | 65°C, 3 days            | 65°C, 5 days            |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| EC <sub>50</sub> | 2.13 x 10 <sup>-9</sup> | 2.15 x 10 <sup>-8</sup> | 1.41 x 10 <sup>-7</sup> | 9.53 x 10 <sup>-7</sup> |

The ADCC Reporter Bioassay (FcγRIIIa-V158) was used in a stability study of Rituximab and CD20+ WIL2-S Target Cells following antibody heat denaturation at 65°C for the indicating number of days.

# ADCC Assay: Ordering information

| Human FcγRIIIa-V Variant ADCC                   | Product Category | Cat. No. | Components  | Assays in 96-well format |
|---|------------------|----------|---|--------------------------|
| ADCC Reporter Bioassay, Complete Kit (Raji)     | Catalog          | G7015    | 1 x 1 vial ADCC Bioassay Effector Cells<br>1 x 1 vial ADCC Bioassay Target Cells (Raji)<br>1 x 5 µg Control Ab, Anti-CD20<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| ADCC Reporter Bioassay, Target Kit (Raji)       | Catalog          | G7016    | 1 x 1 vial ADCC Bioassay Target Cells (Raji)<br>1 x 5 µg Control Ab, Anti-CD20  | 600                      |
| ADCC Reporter Bioassay, Core Kit                | Catalog          | G7010    | 1 x 1 vial ADCC Bioassay Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System   | 120                      |
| ADCC Reporter Bioassay, Core Kit 5X             | Catalog          | G7018    | 5 x 1 vial ADCC Bioassay Effector Cells<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System   | 600                      |
| ADCC Bioassay Effector Cells, Propagation Model | Catalog          | G7102    | 2 x 1 vial ADCC Bioassay Effector Cells (2x10 <sup>7</sup> /ml)   |                          |

| Human FcγRIIIa-F Variant ADCC                              |         |       |  |     |
|--|---------|-------|--|-----|
| ADCC Reporter Bioassay, F Variant, Core Kit                | Catalog | G9790 | 1 x 1 vial ADCC Bioassay Effector Cells, F Variant<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| ADCC Reporter Bioassay, F Variant, Core Kit 5X             | Catalog | G9798 | 5 x 1 vial ADCC Bioassay Effector Cells, F Variant<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| ADCC Bioassay Effector Cells, F Variant, Propagation Model | Catalog | G9302 | 2 x 1 vial ADCC Bioassay Effector Cells, F Variant (2x10 <sup>7</sup> /ml)   |     |

| FcγR Effector Bioassay Bundles                                     |     |           |   |         |
|--|-----|-----------|---|---------|
| Human FcγR Bioassay 5-Pack (includes catalog & CAS Effector Cells) |     |           |   |         |
| Human FCγR Bioassay 5-Pack   | CAS | CS1781D02 | 1 x 1 vial FcγRIIIa-V158 Effector Cells (catalog)<br>1 x 1 vial FcγRIIIa-F158 Effector Cells (catalog)<br>1 x 1 vial FcγRIIIa-H131 Effector Cells (catalog)<br>1 x 1 vial FcγRIIIa-R131 Effector Cells (CAS)<br>1 x 1 vial FcγRI Effector Cells (CAS)<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 5 x 120 |



| Mouse Fc $\gamma$ RIV ADCC  | Product Category | Cat. No. | Components   | Assays in 96-well format |
|---|------------------|----------|--|--------------------------|
| mFc $\gamma$ RIV ADCC Reporter Bioassay, Complete Kit                             | Catalog          | M1201    | 1 x 1 vial mFc $\gamma$ RIV Effector Cells<br>1 x 1 vial Target Cells (Raji)<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System<br>1 x 1 vial Control Ab, Anti-CD20 | 120                      |
| mFc $\gamma$ RIV ADCC Reporter Bioassay, Core Kit                                 | Catalog          | M1211    | 1 x 1 vial mFc $\gamma$ RIV Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System   | 120                      |
| mFc $\gamma$ RIV ADCC Reporter Bioassay, Core Kit 5X                              | Catalog          | M1215    | 5 x 1 vial mFc $\gamma$ RIV Effector Cells 5X<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System  | 600                      |
| mFc $\gamma$ RIV ADCC Bioassay Effector Cells, Fc $\gamma$ RIV, Propagation Model | Catalog          | M1212    | 2 x 1 vial mFc $\gamma$ RIV Effector Cells (2x10 <sup>7</sup> /ml)   |                          |

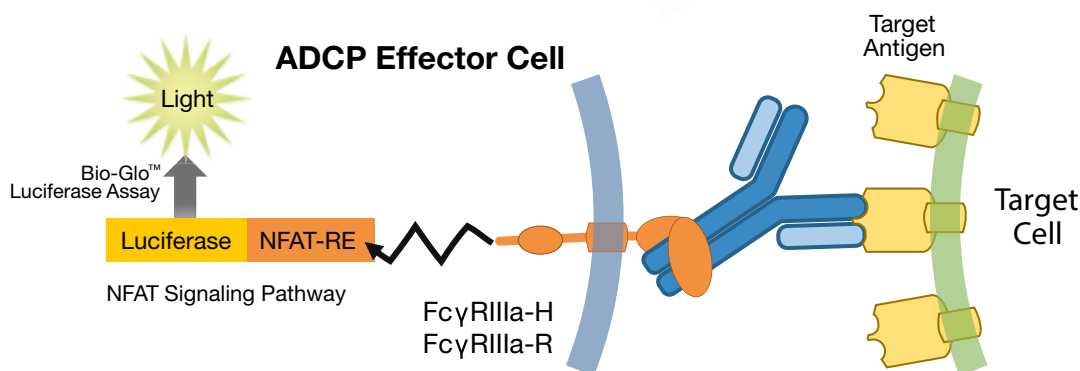
| Mouse Fc $\gamma$ RIII ADCC                                       |     |           |  |     |
|---|-----|-----------|--|-----|
| mFc $\gamma$ RIII ADCC Reporter Bioassay, Core Kit                | CAS | CS1779B08 | 1 x 1 vial mFc $\gamma$ RIII Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 mL RMP 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| mFc $\gamma$ RIII ADCC Bioassay Effector Cells, Propagation Model | CAS | CS1779B06 | 2 x 1 vial mFc $\gamma$ RIII Effector Cells (2x10 <sup>7</sup> /ml)  |     |

| Bio-Glo™ Reagents                |         |       |   |      |
|----------------------------------|---------|-------|---|------|
| Bio-Glo™ Luciferase Assay System | Catalog | G7941 | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate  | 100  |
| Bio-Glo™ Luciferase Assay System | Catalog | G7940 | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate | 1000 |

| Other Target Cells for use with ADCC Bioassays  |     |          |  |     |
|---|-----|----------|--|-----|
| Anti-TNF $\alpha$ ADCC Bioassays  |     |          |  |     |
| Membrane TNF $\alpha$ Bioassay<br>Cross-listed in the Growth Factor & Cytokine chapter                    | CAS | CS185502 | 1 x 1 vial Membrane TNF $\alpha$ CHO-K1 Target Cells | 120 |
| Membrane TNF $\alpha$ Bioassay, Propagation Model<br>Cross-listed in the Growth Factor & Cytokine chapter | CAS | CS185501 | 2 x 1 vial Membrane TNF $\alpha$ CHO-K1 Target Cells |     |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# Antibody-Dependent Cell-Mediated Phagocytosis (ADCP) Assays



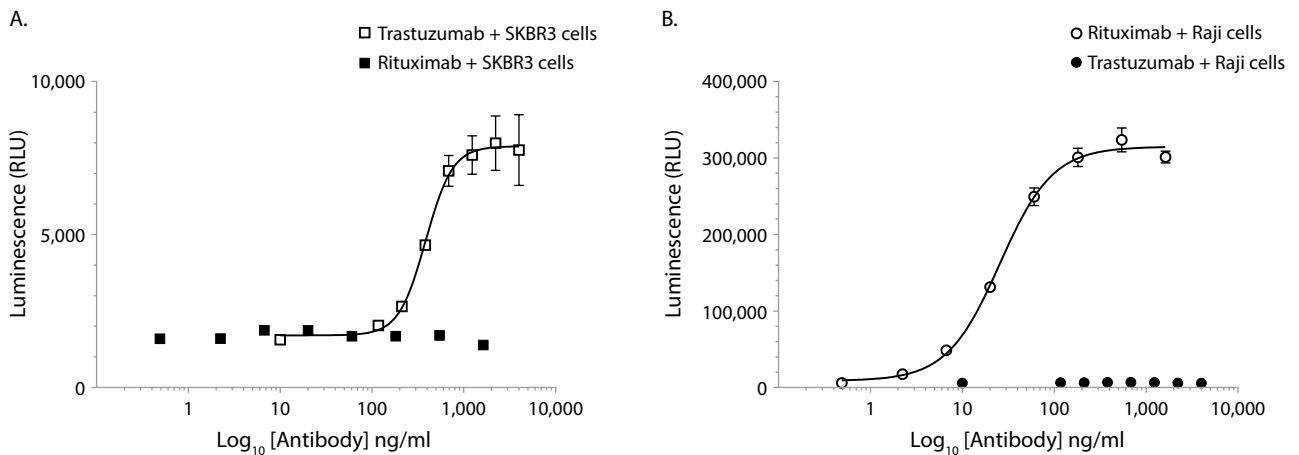
**ADCP assay principle:** The human ADCP Reporter Bioassays are biologically relevant MOA-based assays that can be used to measure the potency and stability of antibodies and other biologics that bind and activate the specific Fc $\gamma$  receptor. The assays consist of Jurkat cells stably expressing human Fc $\gamma$  and NFAT-induced luciferase.

Antibody-dependent cell-mediated phagocytosis (ADCP) is an important mechanism of action (MOA) of therapeutic antibodies designed to recognize and mediate the elimination of virus-infected or diseased (e.g. tumor) cells. Unlike antibody-dependent cell-mediated cytotoxicity (ADCC), which is mediated primarily through Fc $\gamma$ RIIIa expressed on NK cells, ADCP can be mediated by monocytes, macrophages, neutrophils and dendritic cells via Fc $\gamma$ RIIIa (CD32a), Fc $\gamma$ RI (CD64) and Fc $\gamma$ RIIIa (CD16a). The Fc $\gamma$ RIIIa-H ADCP Reporter Bioassay is a bioluminescent cell-based assay that overcomes the limitations of existing assays. It can be used to measure the potency and stability of antibodies and other biologics with Fc domains that specifically bind and activate Fc $\gamma$ RIIIa. The assay consists of a genetically engineered Jurkat T cell line that expresses the high affinity Fc $\gamma$ RIIIa-H variant that contains a histidine (H) at amino acid 131, and a luciferase reporter driven by an NFAT-response element (NFAT-RE). Compared to the low-affinity Fc $\gamma$ RIIIa-R variant, that contains an arginine (R) at amino acid 131 Fc $\gamma$ RIIIa-H exhibits higher affinity for IgG2 isotypes. The ADCP Effector Cells are provided in a “thaw-and-use” format, which includes cryopreserved cells that can be thawed, plated and used in an assay.

## Bioassay Kit Formats

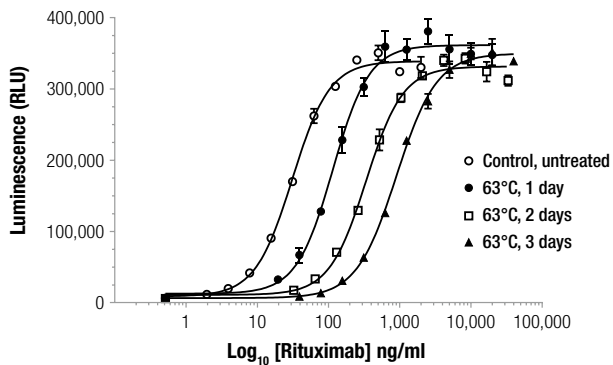
- Core Kits: include ADCC Bioassay Effector Cells, Medium, Low-IgG Serum, Bio-Glo™ Luciferase Reagent Recommended for routine use with customer-defined antibody and target cells.
- Complete Kits: include all Core Kit components, Target Cells, control antibody Recommended for use as a starter kit.

## ADCP Bioassay specificity



The assay shows high specificity as demonstrated with SKBR-3 (Her2+) or frozen Raji (CD20+) target cells. Addition of anti-Her2 Trastuzumab or anti-CD20 Rituximab in combination with the appropriate antigen-expressing target cells gives an assay response, whereas no response is obtained when the antibody cannot bind to target cells.

## Stability indicating



Samples of rituximab (anti-CD20) were maintained at 4°C (control) or heat-denatured at 63°C for the indicated times and analyzed using the FcγRIIa-H ADCP Reporter Bioassay. The EC<sub>50</sub> values were 32 ng/ml for the control, and 116 ng/ml, 339 ng/ml and 904 ng/ml across the three time points.

## Recent Citations

1. 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.
2. Crosby, E.J., et al. (2018) Complimentary mechanisms of dual checkpoint blockade expand unique T-cell repertoires and activate adaptive anti-tumor immunity in triple-negative breast tumors. *OncolImmunology* 7, e1421891.

# ADCP Assay: Ordering information

| Human FcγRIIa-H Variant ADCP                              | Product Category | Cat. No. | Components   | Assays in 96-well format |
|---|------------------|----------|--|--------------------------|
| FcγRIIa-H ADCP Reporter Bioassay, Complete Kit            | Catalog          | G9901    | 1 x 1 vial FcγRIIa-H Effector Cells<br>1 x 1 vial Target Cells (Raji)<br>1 x 2.5 μg ADCP Control Ab, Anti-CD20<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| FcγRIIa-H ADCP Reporter Bioassay, Core Kit                | Catalog          | G9991    | 1 x 1 vial FcγRIIa-H Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System  | 120                      |
| FcγRIIa-H ADCP Reporter Bioassay, Core Kit 5X             | Catalog          | G9995    | 5 x 1 vial FcγRIIa-H Effector Cells 5X<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System   | 600                      |
| FcγRIIa-H ADCP Bioassay Effector Cells, Propagation Model | Catalog          | G9871    | 2 x 1 vial FcγRIIa-H Effector Cells (CPM)  |                          |

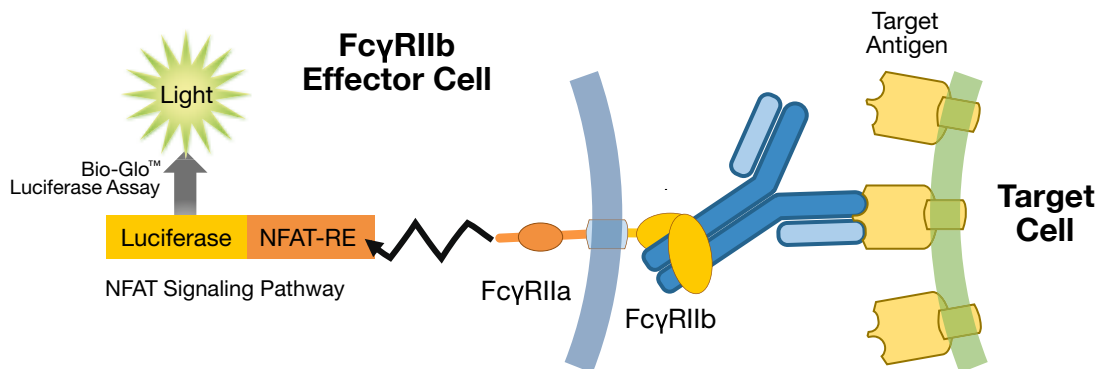
| Human FcγRIIa-R Variant ADCP                              |     |           |   |     |
|---|-----|-----------|---|-----|
| FcγRIIa-R ADCP Reporter Bioassay, Core Kit                | CAS | CS1781B08 | 1 x 1 vial FcγRIIa-R Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| FcγRIIa-R ADCP Bioassay Effector Cells, Propagation Model | CAS | CS1781B06 | 2 x 1 vial FcγRIIa-R Bioassay Effector Cells (2x10 <sup>7</sup> /ml)  |     |

| Human FcγRI Variant ADCP                              |     |           |   |     |
|---|-----|-----------|---|-----|
| FcγRI ADCP Reporter Bioassay, Core Kit                | CAS | CS1781C08 | 1 x 1 vial FcγRI Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| FcγRI ADCP Reporter Bioassay, Core Kit 5X             | CAS | CS1781C10 | 5 x 1 vial FcγRI Effector Cells<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo Luciferase Assay System  | 600 |
| FcγRI ADCP Bioassay Effector Cells, Propagation Model | CAS | CS1781C06 | 2 x 1 vial FcγRI ADCP Bioassay (2x10 <sup>7</sup> /ml)  |     |

| Bio-Glo™ Reagents                |         |       |   |      |
|----------------------------------|---------|-------|---|------|
| Bio-Glo™ Luciferase Assay System | Catalog | G7941 | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate  | 100  |
| Bio-Glo™ Luciferase Assay System | Catalog | G7940 | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate | 1000 |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

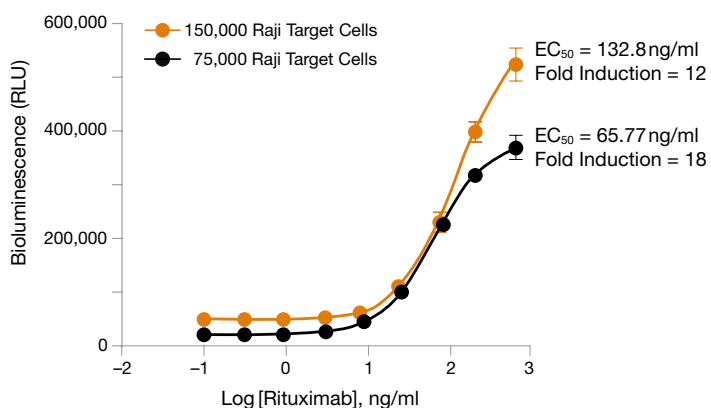
# Human Fc $\gamma$ RIIb Bioassay



**Fc $\gamma$ RIIb assay principle:** Activation of Fc $\gamma$ RIIb transmits a signal through the Fc $\gamma$ RIIa domain to drive a luciferase reporter through an NFAT response element (NFAT-RE). In the presence of antibody and target cells expressing the relevant antigen, effector cells expressing Fc $\gamma$ RIIb/RIIa chimera will transduce intracellular signals resulting in NFAT-mediated luciferase activity that can be easily quantified.

The human Fc $\gamma$ RIIb Bioassay measures Fc $\gamma$ RIIb activation through chimeric Fc $\gamma$ RIIb/IIa receptor. Fc $\gamma$ RIIb is an inhibitory receptor; however, the receptor engineered into this bioassay is a chimeric receptor containing the extracellular domain of the inhibitory Fc $\gamma$ RIIb receptor and the intracellular domain of the activating Fc $\gamma$ RIIa-H receptor, to provide an activating signal upon antibody binding to Fc $\gamma$ RIIb. Fc $\gamma$ RIIb Effector Cells consist of Jurkat cells engineered to express the chimeric Fc $\gamma$ RIIb/Fc $\gamma$ RIIa receptor.

## Activation of Fc $\gamma$ RIIb/Fc $\gamma$ RIIa Chimera by Rituximab



The human Fc $\gamma$ RIIb Bioassay is activated by the presence of Rituximab. The assay response is dependant on the number of co-cultured antigen expressing target cells. A robust assay window is achieved with a few as 75,000 target cells.



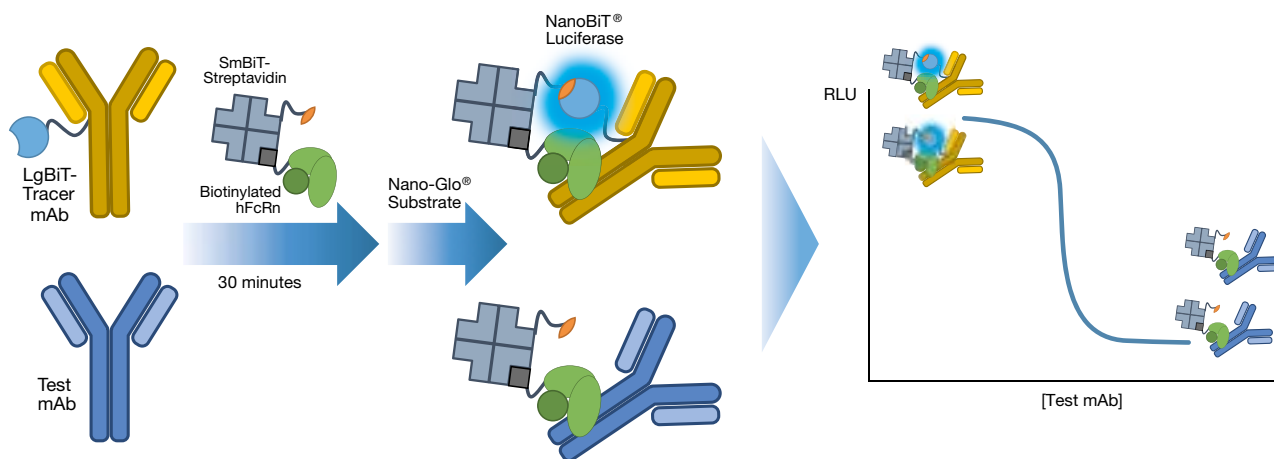
# Human FcγRIIb Bioassay: Ordering information

| Human FcγRIIb Bioassay                             | Product Category | Cat. No.  | Components  | Assays in 96-well format |
|--|------------------|-----------|---|--------------------------|
| FcγRIIb Bioassay, Core Kit                         | CAS              | CS1781E02 | 1 x 1 vial FcγRIIb Effector Cells<br>1 x 4 ml Low IgG Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| FcγRIIb Bioassay, Core Kit 5X                      | CAS              | CS1781E04 | 5 x 1 vial FcγRIIb Effector Cells<br>5 x 4 ml Low IgG Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| FcγRIIb Bioassay Effector Cells, Propagation Model | CAS              | CS1781E07 | 2 x 1 vial FcγRIIb Effector Cells   |                          |

| Bio-Glo™ Reagents                |         |       |   |      |
|----------------------------------|---------|-------|---|------|
| Bio-Glo™ Luciferase Assay System | Catalog | G7941 | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate  | 100  |
| Bio-Glo™ Luciferase Assay System | Catalog | G7940 | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate | 1000 |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# Lumit™ FcRn Binding Immunoassay

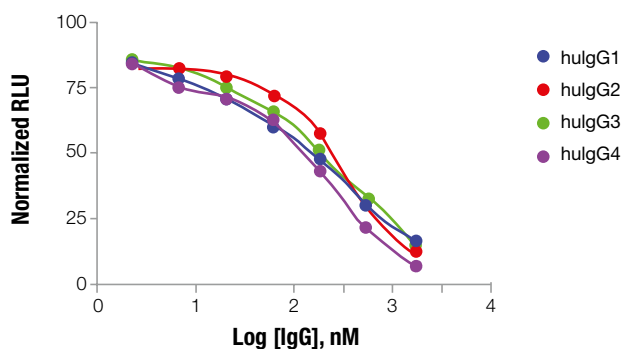


## NanoBiT FcRn competition assay principle

NanoLuc® Binary Technology (NanoBiT®) is a structural complementation reporter designed for biomolecular interaction studies. The NanoBiT® system is composed of two subunits, Large BiT (LgBiT; 18 kDa) and Small BiT (SmBiT; 11 amino acid peptide) that have been optimized for stability and minimal self-association. When two proteins labeled with these subunits come in close proximity, the subunits come together to form an active luciferase enzyme and generate a bright luminescent signal. The small sizes of the NanoBiT® complementation partners minimize interference with protein functionality, and the bright signal allows very sensitive detection.

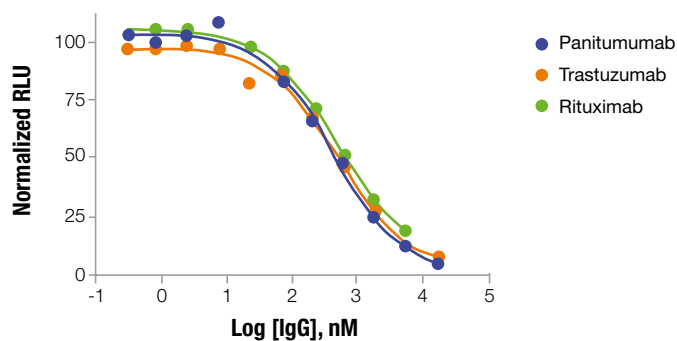
Lumit™ FcRn Binding Immunoassay is a homogeneous (no-wash) competition assay to measure the interaction between human FcRn and Fc proteins including antibodies. Human IgG labeled with LgBiT (hIgG-LgBiT) is used as a tracer. A C-terminus biotinylated FcRn attached to Streptavidin-SmBiT (FcRn-SAv-SmBiT) is used as a target. In the presence of a sample containing no IgG, hIgG-LgBiT tracer binds to the FcRn-SAv-SmBiT target resulting in maximum luminescence signal. For samples containing IgG, unlabeled IgG will compete with tracer for binding to the target, resulting in concentration dependent decrease in luminescent signal.

## Works with All Human IgG Isotypes



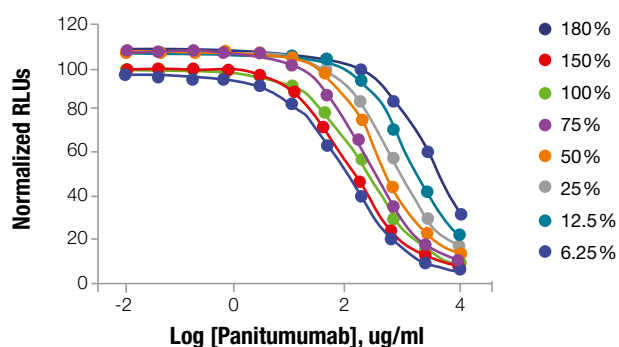
The Lumit™ FcRn Binding Immunoassay can detect FcRn binding affinity for the four major types of human antibody isotypes.

## hFcRn Competition Assay



The affinity of a variety of on-market therapeutic antibodies for FcRn can be detected using the Lumit™ FcRn Binding Immunoassay.

## hFcRn Relative Potency Determination



Dose response curves for Panitumumab-FcRn binding using the Lumit™ FcRn Binding Immunoassay, plotted versus nominal (100%) concentration.

## Lumit™ FcRn Binding Immunoassay: Ordering Information

| Lumit™ FcRn Binding Immunoassay | Product Category | Cat. No. | Components   | Assays in 96-well format |
|---------------------------------|------------------|----------|--|--------------------------|
| Lumit™ FcRn Binding Immunoassay | Catalog          | W1151    | 1 x Biotinylated Human FcRn<br>1 x Streptavidin-SmBiT<br>1 x Tracer-LgBiT Antibody (hIgG1)<br>1 x FcRn Assay Buffer<br>1 x pH Adjustment Buffer<br>1 x Lumit™ Detection Substrate A<br>1 x Positive Control Antibody (hIgG1) | 96                       |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# T Cell Activation Bioassays

## Benefits

- ✓ Platform to enable development of anti-CD3 bispecific molecules
- ✓ Useful for discovery and development of CAR-T cell therapies

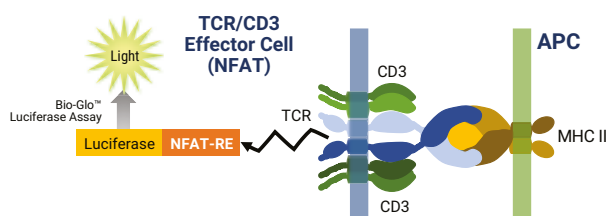
## Overview

Immunotherapy strategies aimed at inducing, strengthening or engineering T cell responses have emerged as promising approaches for the treatment of diseases such as cancer and autoimmunity. T cell activation is initiated by engagement of the TCR/CD3 complex and the co-stimulatory receptor CD28. TCR/CD3 engagement activates the NFAT pathway and TCR/CD3 + CD28 co-engagement activates NFAT, AP-1 and NF- $\kappa$ B pathways, thereby inducing IL-2 production.

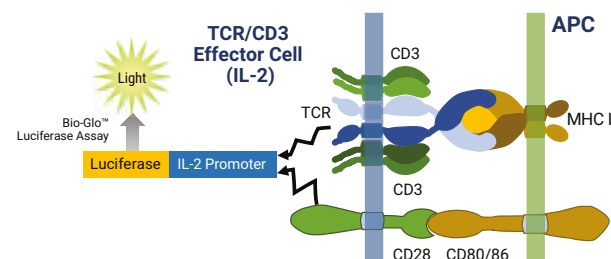
The T Cell Activation Bioassays are bioluminescent cell-based assays that overcome limitations of inconsistencies posed by existing assays. They can be used for the discovery and development of anti-CD3 bispecific molecules and to evaluate the activity of exogenously expressed chimeric antigen receptor (CAR) constructs. The assays consist of Jurkat T cells genetically engineered to express luciferase downstream of either NFAT or IL-2 response elements.

The bioassay workflow is simple, robust and compatible with 96-well and 384-well plate formats. Additionally, the bioassay is tolerant to human serum, indicating potential for further development into a neutralizing antibody bioassay.

### T Cell Activation Bioassay (NFAT)

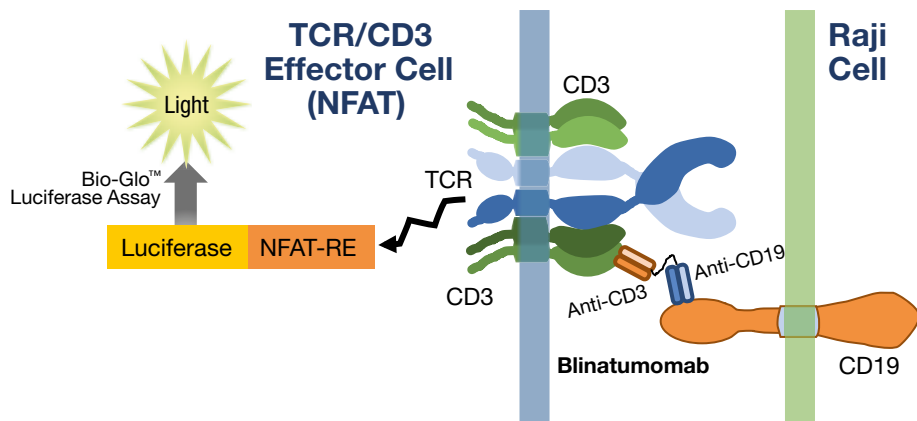


### T Cell Activation Bioassay (IL-2)



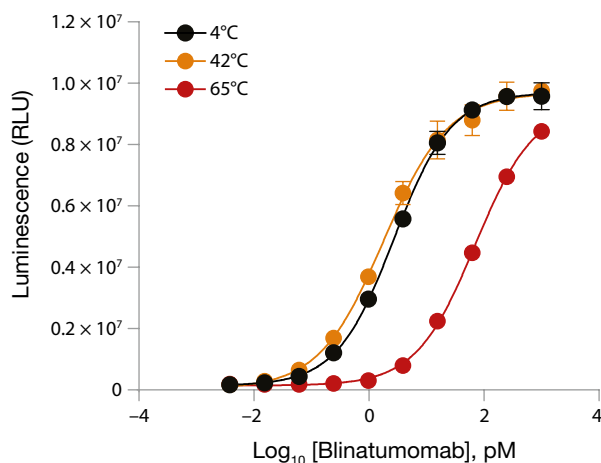
**T Cell Activation Bioassay principle.** The assay consists of a genetically engineered Jurkat T cell line that expresses a luciferase reporter (TCR/CD3 Effector Cells) driven by either an NFAT-response element (NFAT-RE) or an IL-2 promoter. When the TCR/CD3 Effector Cells (NFAT) are engaged with an appropriate TCR/CD3 ligand or anti-TCR/CD3 antibody, the TCR transduces intracellular signals, resulting in NFAT-RE-mediated luminescence. Similarly, when the TCR/CD3 Effector Cells (IL-2) are co-engaged with an anti-TCR/CD3 and an anti-CD28 stimulus, receptor-mediated signaling results in IL-2 promoter-mediated luminescence.

**The T Cell Activation Bioassay (NFAT or IL-2) can be used to measure the activity and specificity of bispecific antibodies**

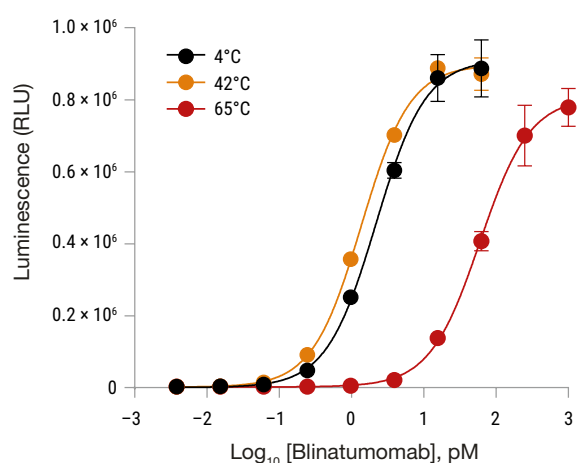


**Measuring Blinatumomab (CD3×CD19 BiTE) activity.** When the TCR/CD3 Effector Cells (NFAT) are engaged with an appropriate TCR/CD3 ligand or anti-TCR/CD3 antibody, the TCR transduces intracellular signals resulting in NFAT-RE-mediated luminescence.

### T Cell Activation Bioassay (NFAT)



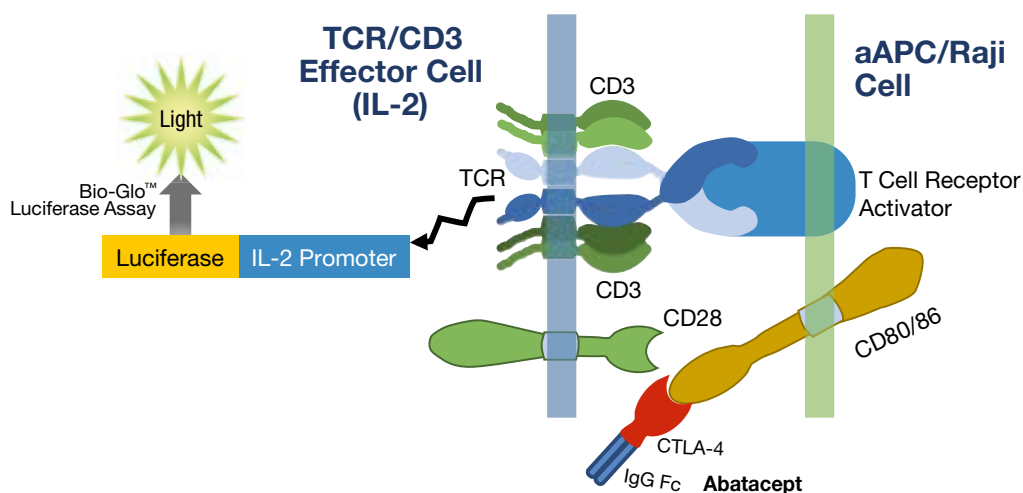
### T Cell Activation Bioassay (IL-2)



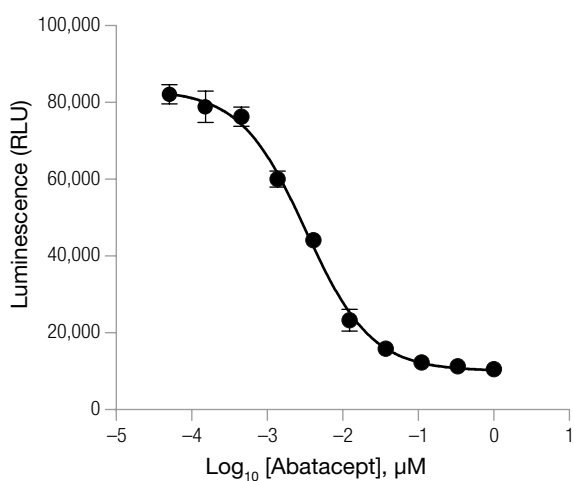
**The T Cell Activation Bioassay indicates the relative stability of antibodies.** Samples of Blinatumomab anti-CD3 and anti-CD19 bispecific antibody were stored at 4°C or heat-treated (42°C or 65°C). The antibodies were analyzed using the T Cell Activation Bioassay (NFAT or IL-2, as indicated).



The T Cell Activation Bioassay (IL-2) can be used to measure the activity of biologic drugs targeting the CD28 signaling pathway

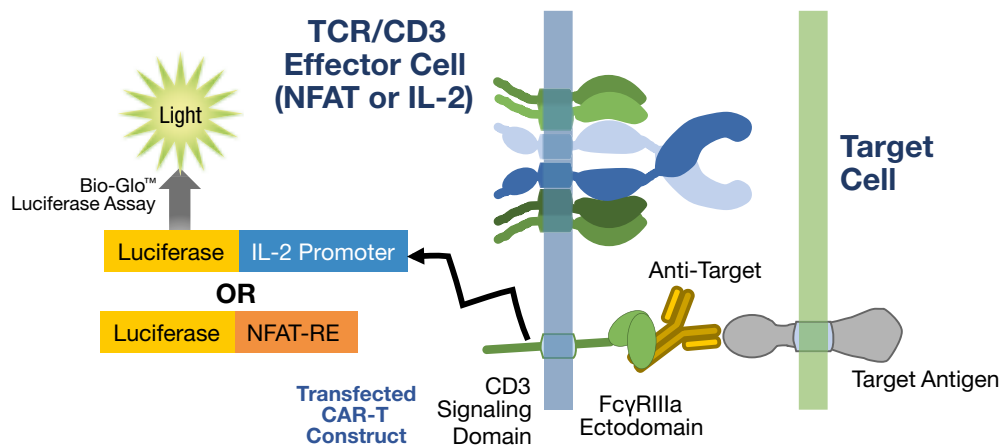


**Blockade of CD28:CD80/86 with TCR engagement.** Abatacept prevents co-stimulation of IL-2 promoter by CD28:CD80/86 engagement



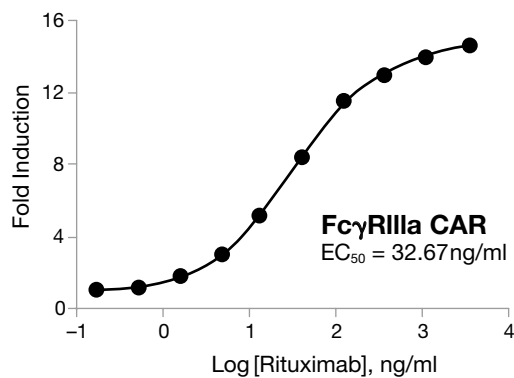
**MOA-reflecting specificity.** TCR/CD3 Effector Cells (IL-2) were incubated with increasing concentrations of Abatacept in the presence of an artificial antigen presenting cell (aAPC) consisting of Raji (CD80/CD86+) cells expressing an engineered cell surface protein designed to activate cognate TCRs in an antigen-dependent manner.

## Measuring the Activity of CAR-T Cell Receptors

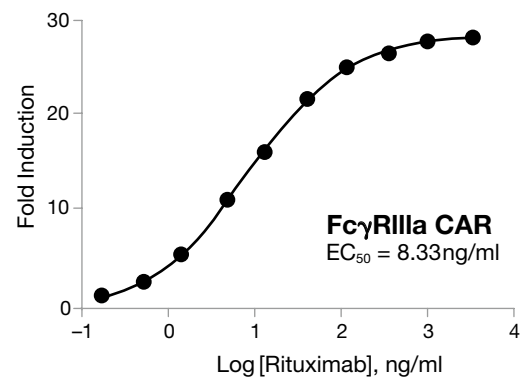


**Measurement of Chimeric Antigen Receptor T (CAR-T) Cell Activity.** TCR/CD3 (NFAT or IL-2) effector cells were transiently transfected with a chimeric antigen receptor.

### TCR/CD3 Effector Cells (NFAT)



### TCR/CD3 Effector Cells (IL-2)



TCR/CD3 (NFAT or IL-2) effector cells were engineered to express FcγRIIIa CAR-T receptors.

# T Cell Activation Bioassays:

## Ordering information

| T Cell Activation Bioassays                          | Product Category | Cat. No.  | Components  | Assays in 96-well format |
|--|------------------|-----------|---|--------------------------|
| T Cell Activation Bioassay (NFAT)                    | Catalog          | J1621     | 1 x 1 vial TCR/CD3 Effector Cells (NFAT)<br>1 x 36 ml RPMI 1640 Medium<br>1 x 4 ml Fetal Bovine Serum<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| T Cell Activation Bioassay (NFAT) 5X                 | Catalog          | J1625     | 5 x 1 vial TCR/CD3 Effector Cells (NFAT)<br>5 x 36 ml RPMI 1640 Medium<br>5 x 4 ml Fetal Bovine Serum<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| T Cell Activation Bioassay (NFAT), Propagation Model | Catalog          | J1601     | 2 x 1 vial TCR/CD3 Effector Cells (NFAT)  |                          |
| T Cell Activation Bioassay (IL-2)                    | Catalog          | J1651     | 1 x 1 vial TCR/CD3 Effector Cells (NFAT)<br>1 x 36 ml RPMI 1640 Medium<br>1 x 4 ml Fetal Bovine Serum<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| T Cell Activation Bioassay (IL-2) 5X                 | Catalog          | J1655     | 5 x 1 vial TCR/CD3 Effector Cells (NFAT)<br>5 x 36 ml RPMI 1640 Medium<br>5 x 4 ml Fetal Bovine Serum<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| T Cell Activation Bioassay (IL-2), Propagation Model | Catalog          | J1631     | 2 x 1 vial TCR/CD3 Effector Cells (NFAT)  |                          |
| T Cell Activation Bioassay (NFkB), Propagation Model | CAS              | CS1979A01 | 2 x 1 vial TCR/CD3 Effector Cells (NFkB)  |                          |

| TCRαγ Knock-Out Cell Lines<br>(NanoLuc® Luciferase) |     |          |                                  |  |
|---|-----|----------|----------------------------------|--|
| TCRαγ-KO (CD4+) Cell Line, Propagation Model        | CAS | CS310102 | 2 x 1 vial TCRαγ-KO (CD4+) Cells |  |
| TCRαγ-KO (CD8+) Cell Line, Propagation Model        | CAS | CS310104 | 2 x 1 vial TCRαγ-KO (CD8+) Cells |  |

| Bio-Glo™ Reagents                   |         |       |   |      |
|-------------------------------------|---------|-------|---|------|
| Bio-Glo™ Luciferase Assay System    | Catalog | G7941 | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate        | 100  |
| Bio-Glo™ Luciferase Assay System    | Catalog | G7940 | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate       | 1000 |
| Bio-Glo-NL™ Luciferase Assay System | Catalog | J3081 | 1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate  | 100  |
| Bio-Glo-NL™ Luciferase Assay System | Catalog | J3082 | 1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate | 1000 |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# Immune Checkpoint Modulation Bioassays

## Benefits

- ✓ Provide quantitative measurements of immune checkpoint blockade or agonist activity
- ✓ Combination bioassays support development of therapeutic approaches targeting more than one immune checkpoint receptor
- ✓ Prequalified according to ICH guidelines

## Overview

The human immune system is comprised of a complex network of co-inhibitory and co-stimulatory pathways that facilitate the elimination of cells expressing foreign antigens while maintaining tolerance to self-antigen. Immune checkpoint pathways are promising immunotherapy targets for the treatment of cancer and autoimmunity. Activation of T cells via direct stimulation of the T cell receptor or by modulating immune checkpoint pathways are two strategies being employed individually and in combination. Immune checkpoint targets include co-inhibitory and co-stimulatory receptors, individually and in combination. Clinical results showed co-engagement of multiple immune receptors, such as immune inhibitory receptors PD-1 and CTLA4 or PD-1 and TIGIT in combination immunotherapy, elicit much better therapeutic outcomes compared with targeting a single immune receptor. These bioluminescent bioassays reflect the mechanism of action of correlating antibody drug candidates and exhibit assay specificity, precision, accuracy, linearity and robustness required for drug potency and stability determination.

### Co-Inhibitory Receptor Bioassays

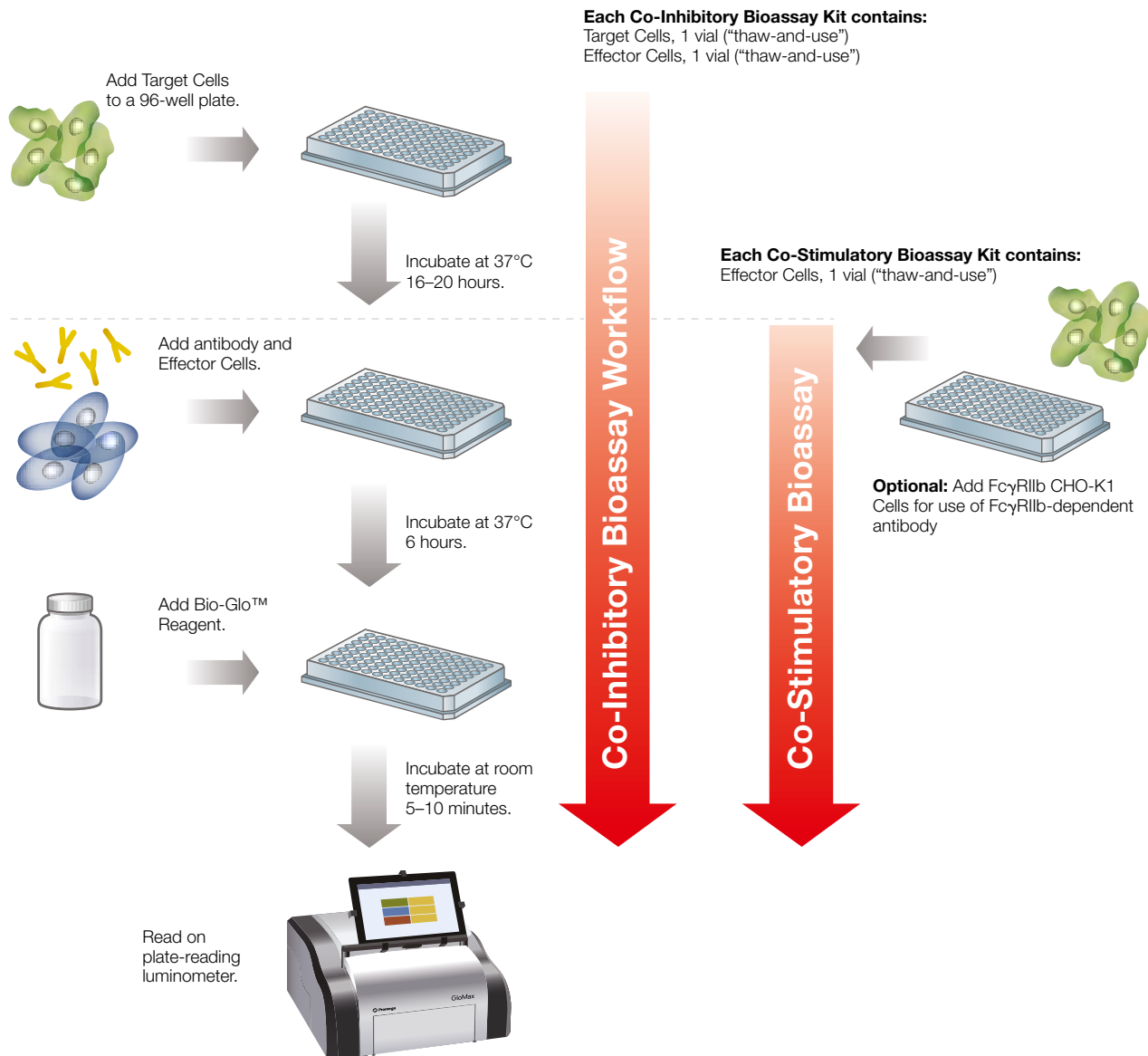
Immune inhibitory receptors (e.g. PD-1, CTLA-4, TIGIT, LAG-3) expressed on activated T cells and B cells play a critical role in regulating immune responses to tumor antigens and autoantigens. Engagement of a receptor by its ligand on an adjacent cell inhibits T cell receptor (TCR) signaling and TCR-mediated proliferation, transcriptional activation and cytokine production. Therapeutic antibodies and Fc-fusion proteins designed to block the receptor-ligand interaction show promising results in clinical trials for the treatment of a variety of cancers.

### Co-Stimulatory Receptor Bioassays

Co-stimulatory immune checkpoint receptors (including GITR, OX40, CD40, 4-1BB and ICOS) are stably expressed in T cell lines. Therapeutic antibodies designed to activate co-stimulatory immune checkpoint receptors are a promising strategy for cancer therapy. Antibody agonist activity can be measured in both the absence and presence of Fc $\gamma$ R-mediated crosslinking, which is therapeutically relevant *in vivo*.

All Bioassays are available as “thaw-and-use” kits in 1X and 5X sizes including all required reagents in standardized formats or as Cell propagation model.

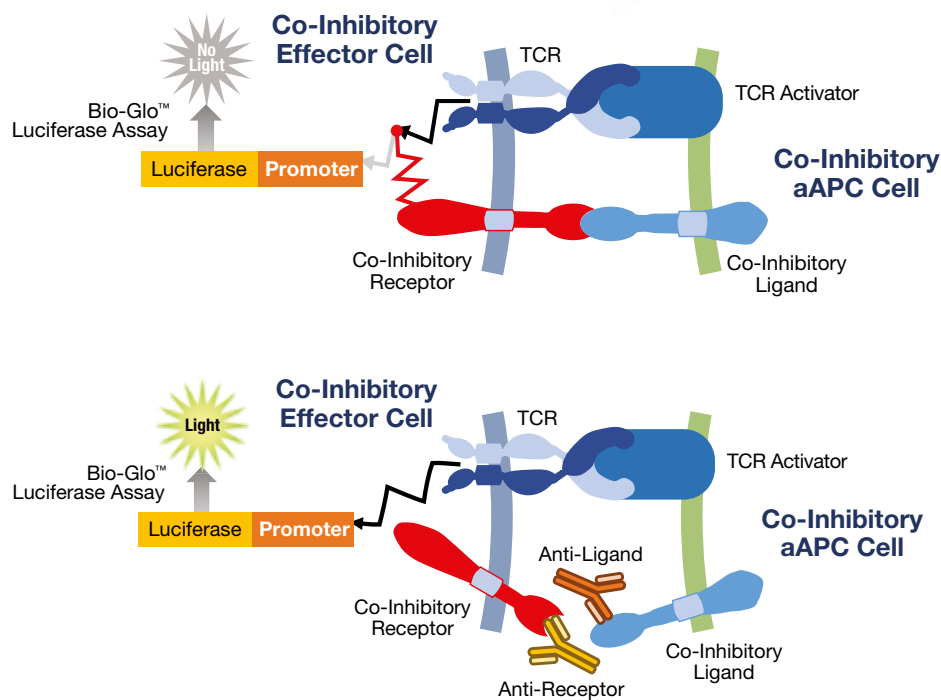
## Schematic protocol for the Immune Checkpoint Bioassays



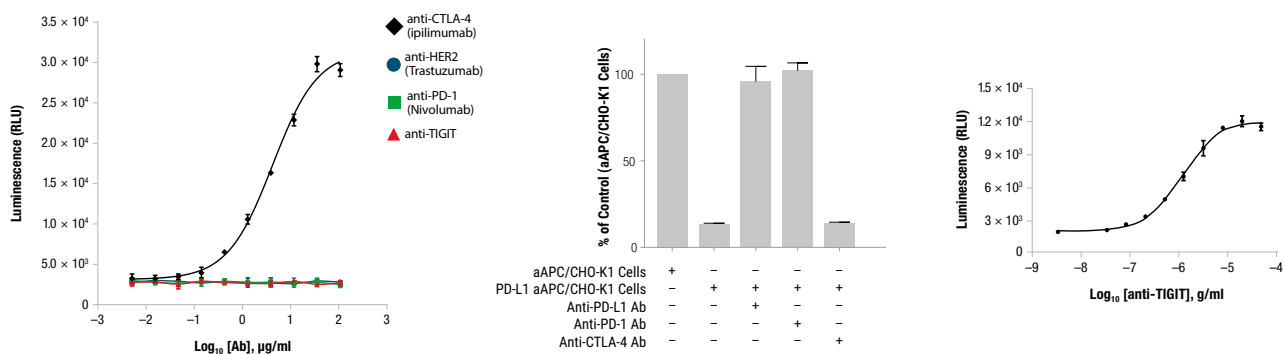
The Immune Checkpoint Bioassays combine a simple, add-mix-read single-day workflow provided in a frozen, "thaw-and-use" format and an optimized protocol that exhibits low variability and high accuracy. No cell culture is required. For some co-stimulatory antibodies it might be beneficial to test the assay together with FcγRIIb CHO-K1 cells initially to check if crosslinking is necessary.



# Co-Inhibitory Receptor Bioassays

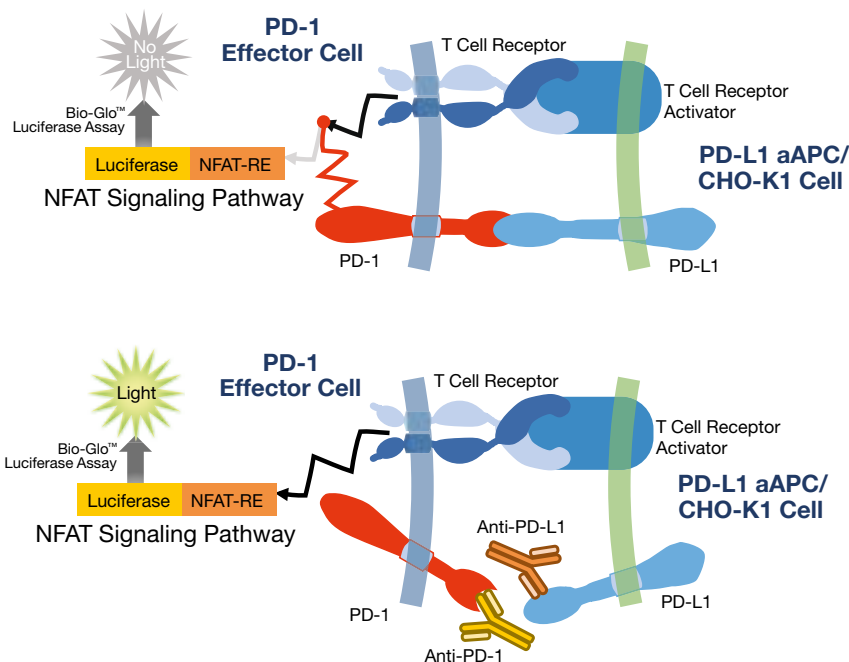


**Co-inhibitory bioassay principle.** The bioassay typically consists of two genetically engineered cell lines: 1) an effector cell that expresses the receptor; and 2) an artificial antigen-presenting cell (aAPC) that expresses the ligand. During co-culture, the TCR is activated by the TCR activator (an engineered cell surface protein) or antigen presentation, and the interaction of ligand with co-inhibitory receptor inhibits TCR signaling. The presence of antibodies blocks the interaction of the co-inhibitory receptor and ligand, which causes removal of the blockade thereby leading to a luciferase signal.



**Antibody potency determination.** Bioassays for CTLA-4 (left), PD-1/PD-L1 (center) and TIGIT/CD155 (right) blockade showed high sensitivity and specificity in measuring the potency of monoclonal antibodies against these co-inhibitory targets.

# PD-1/PD-L1 Blockade Bioassay



**Blockade of PD-1:PD-L1 engagement.** The bioassay consists of two genetically engineered cell lines, PD-1 Effector Cells and PD-L1 aAPC/CHO-K1 Cells. When co-cultured, the TCR is activated but the PD-1/PD-L1 interaction inhibits TCR-mediated luciferase expression. When the PD-1/PD-L1 interaction is disrupted, regained TCR activation induces luminescence (via activation of the NFAT pathway).

The PD-1/PD-L1 Blockade Bioassay is prequalified according to ICH guidelines and shows the precision, accuracy and linearity required for routine use in potency and stability studies. In addition, the bioassay workflow is simple and robust and compatible with both 96-well and 384-well plate formats used for antibody screening in early drug discovery. Finally, the bioassay can be used with up to 10% human serum with minimal impact on anti-PD-1 and anti-PD-L1 EC<sub>50</sub> and fold induction indicating potential for further development into a neutralizing antibody bioassay.

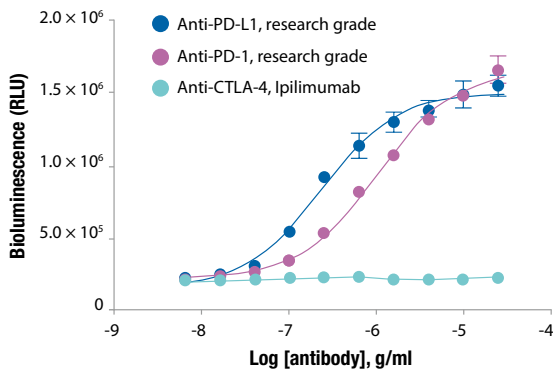
## The assay consists of two genetically engineered cell types:

- **PD-1 Effector Cells:** Jurkat T cells stably expressing human PD-1 and NFAT-induced luciferase.
- **PD-L1 aAPC/CHO-K1 Cells:** CHO-K1 cells stably expressing human PD-L1 and a cell surface protein designed to activate cognate TCRs in an antigen-independent manner.

## Recent Citations:

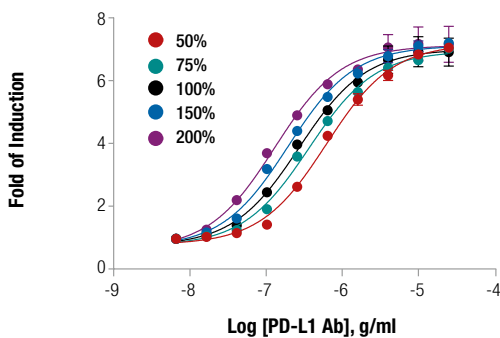
1. Finlay, W.J.J., et al. (2019) Anti-PD1 'SHR-1210' aberrantly targets pro-angiogenic receptors and this polyspecificity can be ablated by paratope refinement. *mAbs*11, 26–44.
2. Boohaker, R.J., et al. (2018) Rational design and development of a peptide inhibitor for the PD-1/PD-L1 interaction. *Cancer Lett.* 434, 11–21.
3. Clarke, A.W., et al. (2018) An anti-TL1A antibody for the treatment of asthma and inflammatory bowel disease. *mAbs*10, 664–77.

## The PD-1/PD-L1 Blockade Bioassay is specific for PD-1 and PD-L1 antibodies



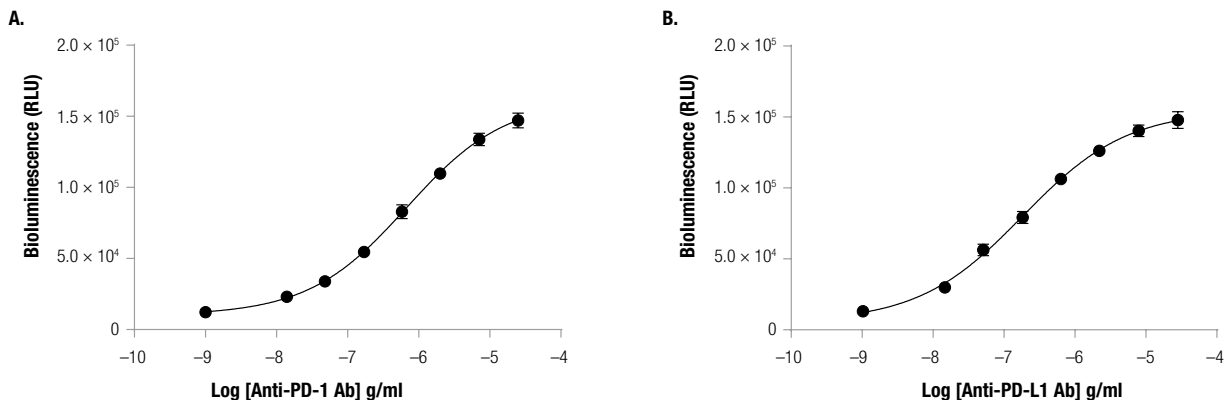
PD-1 Effector Cells and PD-L1 aAPC Cells were incubated for 6 hrs at 37°C with increasing concentrations of either an anti-PD-1, PD-L1 or CTLA-4 antibody. The anti-PD-1 and PD-L1 antibodies, but not the anti-CTLA-4 antibody, blocked the immune checkpoint inhibitory signal resulting in luciferase activity.

## The PD-1/PD-L1 Blockade Bioassay is accurate and reproducible over a relative potency range of 50–200 %



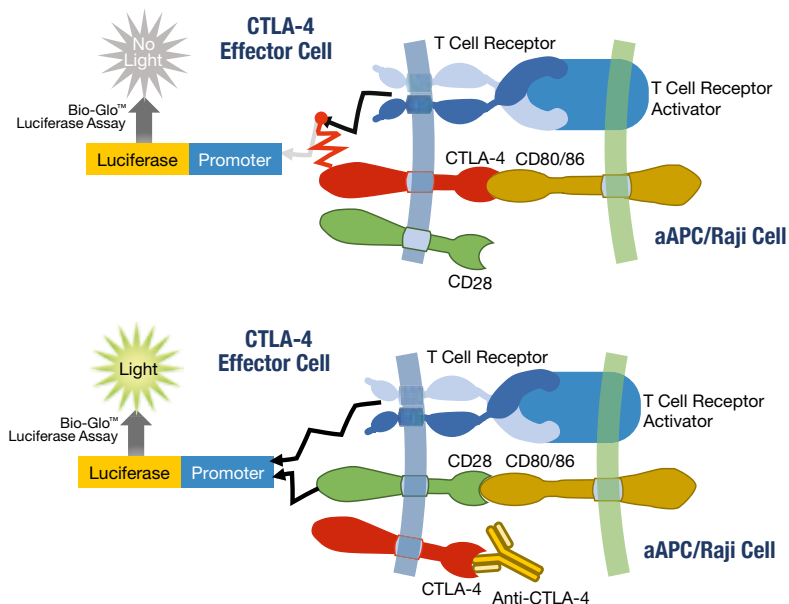
PD-1 Effector Cells and PD-L1 aAPC Cells were incubated with increasing concentrations of anti-PD-L1 antibody preparations representing a 50–200 % potency range. The PD-1/PD-L1 Blockade Bioassay demonstrated appropriate rank ordering of antibody potency.

## The assay is amenable to 384-well plate format and compatible with automation



Anti-PD-1 (Panel A) or anti-PD-L1 Ab (Panel B) was tested in the PD-1/PD-L1 Blockade Bioassay with a Multidrop™ Combi nL (Thermo Scientific) and Tecan Freedom EVO® 200 with Multichannel Arm™ 384.

# CTLA-4 Blockade Bioassay



**Blockade of CTLA4:CD80/86 engagement.** When the two cell types are co-cultured, CTLA-4 competes with CD28 for their shared ligands, CD80 and CD86, and thus inhibits CD28 pathway activation and promoter-mediated luminescence. Addition of an anti-CTLA-4 antibody blocks the interaction of CTLA-4 with its ligands CD80 and CD86 and results in promoter-mediated luminescence.

CTLA-4, also known as CD152, is an immune inhibitory receptor constitutively expressed on regulatory T cells and upregulated in activated T cells. CTLA-4 plays a critical role in regulating immune responses to tumor antigens and autoantigens. When CTLA-4 expression is upregulated on the surface of T cells, the T cells bind B7 (CD80/86) with a higher affinity, and thus out-compete the positive co-stimulatory signal from CD28. In addition, engagement of CTLA-4 by either of its ligands, CD80 (B7-1) or CD86 (B7-2), on an adjacent antigen presenting cell (APC) inhibits CD28 co-stimulation of T cell activation, cell proliferation and cytokine production. The CTLA-4 Blockade Bioassay reflects the mechanism of action (MOA) of biologics designed to block the interaction of CTLA-4 with its ligands, CD80 and CD86.

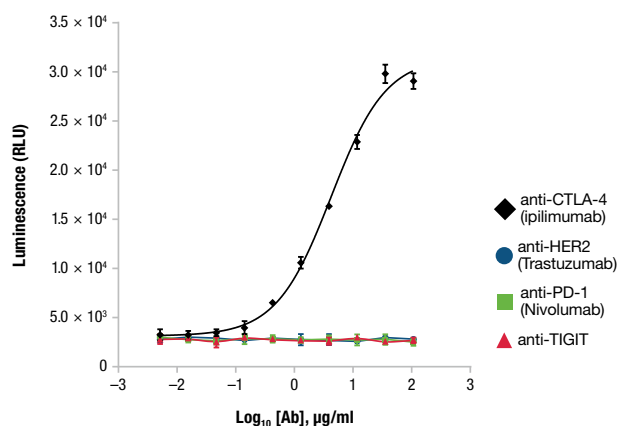
## The assay consists of two genetically engineered cell types:

- **CTLA-4 Effector Cells:** Jurkat T cells stably expressing human CTLA-4 and a luciferase reporter driven by a native promoter which responds to TCR/CD28 activation.
- **aAPC/ Raji Cells:** Raji cells expressing an engineered cell surface protein designed to activate cognate TCRs in an antigen-independent manner and endogenously expressing CTLA-4 ligands CD80 and CD86.

## Recent Citations:

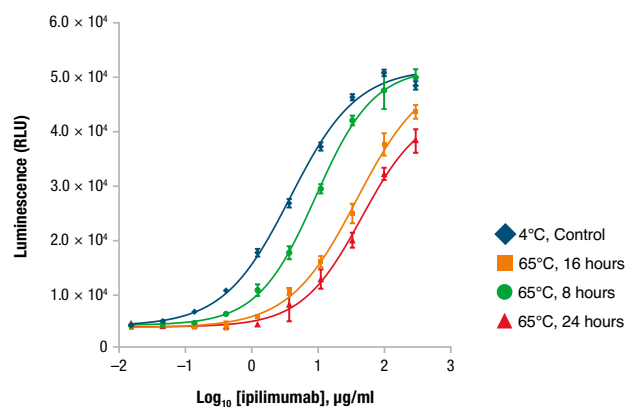
1. Duperret, E.K., et al. (2018) Synthetic DNA-encoded monoclonal antibody delivery of anti-CTLA-4 antibodies induces tumor shrinkage *in vivo*. *Cancer Res.* 78, 6363–70.
2. Gombos, R.B., et al. (2018) Toxicological and pharmacological assessment of AGEN1884, a novel human IgG1 anti-CTLA-4 antibody. *PLoS ONE* 13, e0191926.
3. Waight, J.D., et al. (2018) Selective FcγR co-engagement on APCs modulates the activity of therapeutic antibodies targeting T cell antigens. *Cancer Cell* 33, 1033–47.

## The CTLA-4 Blockade Bioassay reflects the MOA and specificity of biologics designed to block the CTLA-4/CD80 and CD86 interaction.



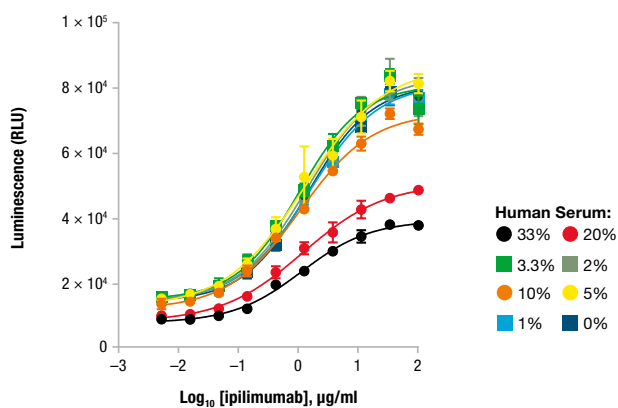
CTLA-4 Effector Cells were incubated with aAPC/Raji Cells in the absence or presence of anti-CTLA-4, anti-PD-1, anti-TIGIT or anti-HER2 blocking antibodies, as indicated.

## Stability Indication



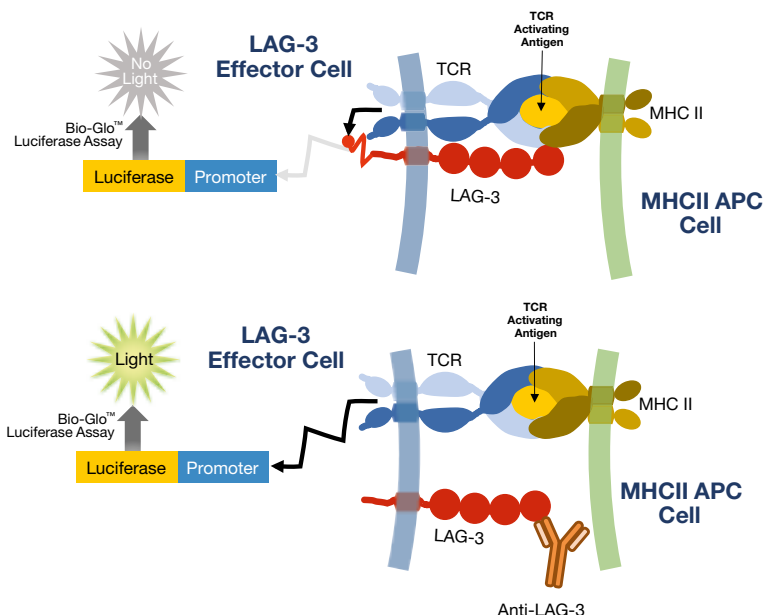
Samples of anti-CTLA-4 antibody, ipilimumab, were maintained at 4°C (control) or heat-denatured at 65°C for the indicated times and analyzed using the CTLA-4 Blockade Bioassay.

## Human serum tolerance



Anti-CTLA-4 blocking antibody, ipilimumab, was analyzed in the absence or presence of increasing concentrations of pooled normal human serum (0–100% in the antibody sample), resulting in final assay concentration of human serum (0–33%).

# LAG-3/MHCII Blockade Bioassay



**Representation of the LAG-3/MHCII Blockade Bioassay.** When co-culturing LAG-3 Effector Cells and MHCII APC Cells, the LAG-3 inhibits TCR pathway-activated luminescence. The addition of anti-LAG-3 antibody blocks LAG-3 binding to MHCII, resulting in full TCR pathway activation, which can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer.

The LAG-3/MHCII Blockade Bioassay is a bioluminescent cell-based assay that measures potency and stability of antibodies and other biologics designed to block the interaction of LAG-3 with its best characterized ligand, major histocompatibility complex II (MHCII). LAG-3, also known as CD223, is an immune checkpoint receptor expressed on activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells and natural killer (NK) cells. Engagement of LAG-3 by MHCII inhibits TCR signaling, cytokine production and proliferation of activated T cells. The LAG-3/MHCII Blockade Bioassay reflects the mechanism of action (MOA) of biologics designed to block the interaction of LAG-3 with its ligand, MHCII.

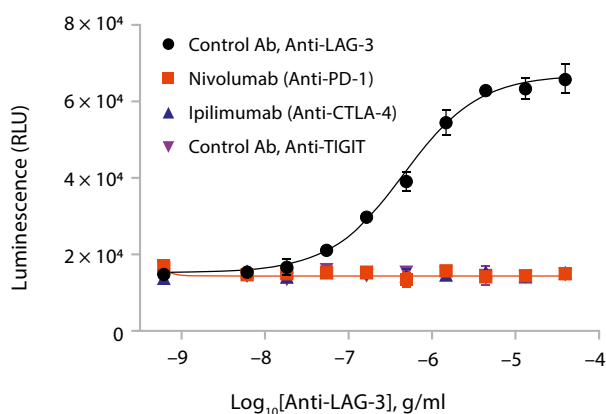
## The assay consists of two genetically engineered cell types:

- **LAG-3 Effector Cells:** Jurkat T cells stably expressing human LAG-3 and a luciferase reporter driven by T cell activation pathway-dependent response elements.
- **MHCII APC Cells:** APC Cells presenting TCR Activating Antigen, a proprietary peptide designed to specifically activate the TCR on the LAG-3 Effector Cells.

## Recent Citations:

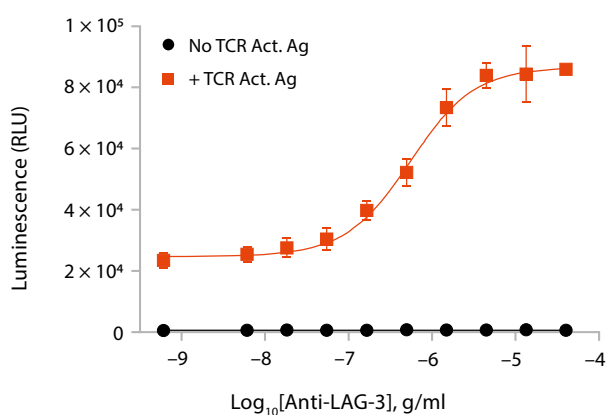
1. Ghosh, S., et al. (2019) TSR 033, a novel therapeutic antibody targeting LAG-3, enhances T cell function and the activity of PD-1 blockade *in vitro* and *in vivo*. Mol. Cancer Ther.

## The LAG-3/MHCII Blockade Bioassay reflects the MOA and specificity of biologics designed to block the LAG-3/MHCII interaction



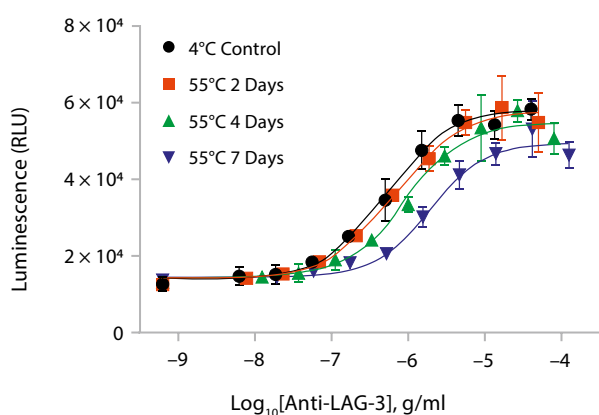
LAG-3 Effector Cells were incubated with TCR Activating Antigen and MHCII APC cells in the presence of anti-LAG-3, anti-PD-1, anti-CTLA-4 or anti-TIGIT antibodies, as indicated.

## The LAG-3/MHCII Blockade Bioassay is dependent on TCR Activating Antigen



MHCII APC Cells were plated with and without TCR Activating Antigen. The next day, a titration of Anti-LAG-3 was plated followed by addition of LAG-3 Effector Cells. After a 5-hour induction, Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover Detection System.

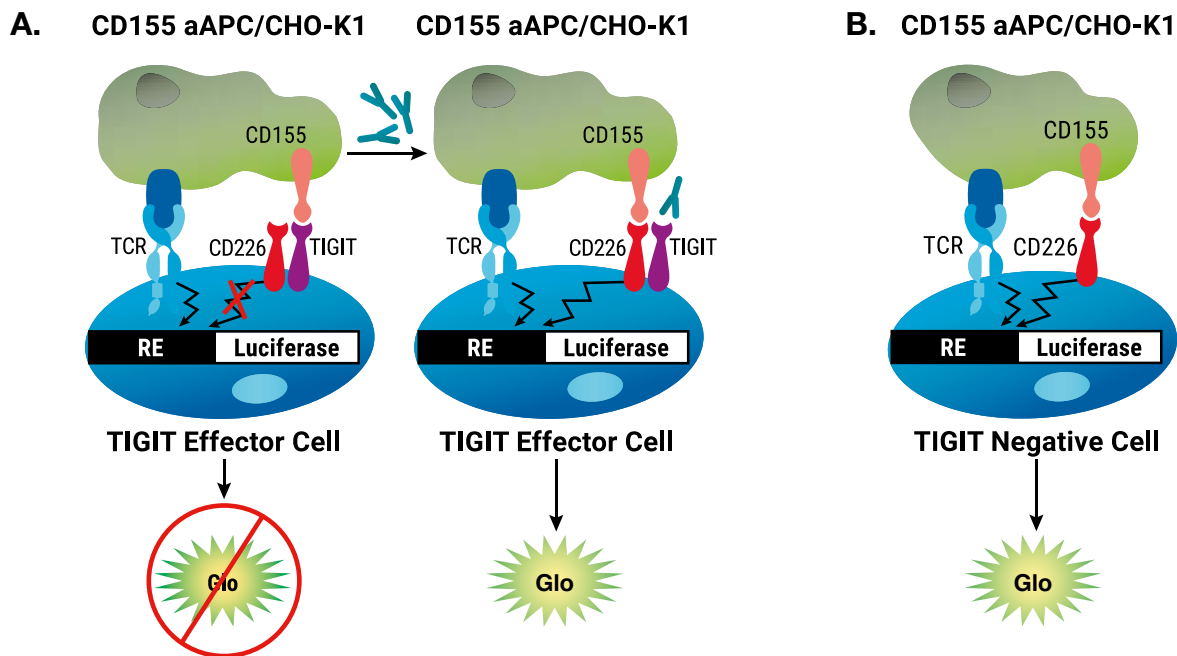
## Stability Indication



Samples of Anti-LAG-3 were maintained at 4°C (control) or heat-denatured at 55°C for the indicated times, then analyzed using the LAG-3/MHCII Blockade Bioassay. Bio-Glo™ Reagent was added and luminescence quantified.



# TIGIT/CD155 Blockade Bioassay



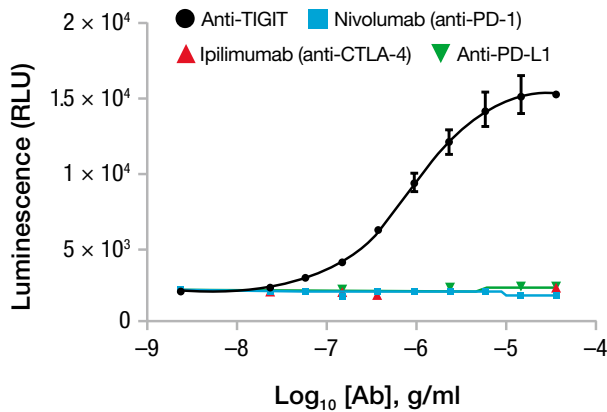
**Representation of the TIGIT/CD155 Blockade Bioassay.** The bioassay consists of two genetically engineered cell lines, TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells. **A.** When co-cultured, TIGIT inhibits CD226 pathway-activated luminescence. The addition of anti-TIGIT antibody blocks the TIGIT/CD155 interaction, thereby re-establishing CD226 pathway-activated luminescence, which can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer. **B.** When co-cultured with non-TIGIT-expressing Effector Cells, CD155 induces luminescence by activation of the CD226 pathway.

TIGIT, also known as WUCAM and VSTM3, is an immune checkpoint protein expressed on lymphocytes. Highest expression levels are observed on effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells, regulatory T cells, and NK cells. TIGIT has several distinct mechanisms of action that inhibit lymphocyte activation. First, TIGIT is an inhibitory counterpart of the co-stimulatory receptor CD226. When TIGIT is present on the surface of lymphocytes, it outcompetes CD226 for CD155 binding and thus negates CD226 signaling. Second, TIGIT inhibits CD226 homodimerization, preventing CD226 signaling. Third, the cytoplasmic tail of TIGIT contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), which could potentially lead to inhibitory signaling. The TIGIT/CD155 Blockade Bioassay reflects the mechanism of action (MOA) of biologics designed to block the TIGIT/CD155 interaction.

**The assay consists of two genetically engineered cell types:**

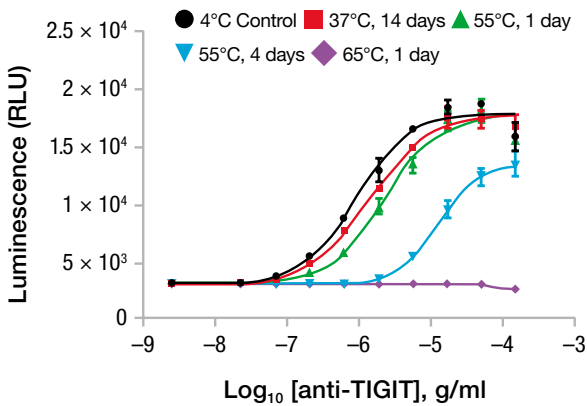
- **TIGIT Effector Cells:** Jurkat T cells expressing human TIGIT with a luciferase reporter driven by a native promoter that can respond to both TCR activation and CD226 co-stimulation.
- **CD155 aAPC/CHO-K1 Cells:** CHO-K1 cells engineered to express human CD155 with an engineered cell-surface protein designed to activate the TCR complex in an antigen-independent manner.

## The TIGIT/CD155 Blockade Bioassay reflects the mechanism of action (MOA) and specificity of biologics designed to block the TIGIT/CD155 interaction



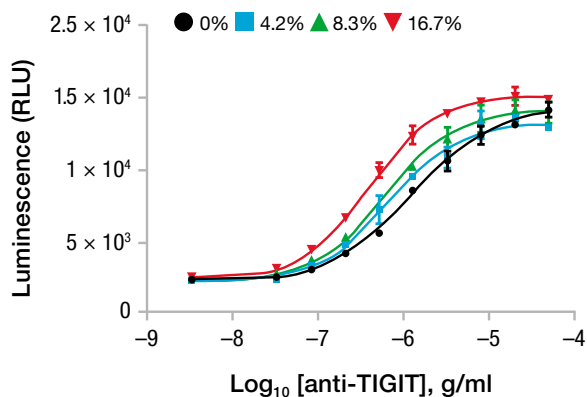
TIGIT Effector Cells were incubated with CD155 aAPC/CHO-K1 Cells in the presence of a serial titration of Anti-TIGIT, anti-PD-1 (Nivolumab), anti-CTLA-4 (Ipilimumab) or anti-PD-L1-blocking antibodies as indicated. Bio-Glo™ Reagent was added and luminescence quantified.

## The TIGIT/CD155 Blockade Bioassay is stability-indicating



Samples of anti-TIGIT, were maintained at 4°C (control) or heat-treated at the indicated temperatures and times, and then analyzed using the TIGIT/CD155 Blockade Bioassay. Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover Detection System.

## The TIGIT/CD155 Blockade Bioassay is tolerant to human serum



Anti-TIGIT, was analyzed in the absence or presence of increasing concentrations of pooled normal human serum (0–100 % in the antibody sample), resulting in final assay concentration of human serum (0–16.7 %). Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover Detection System.

# Co-Inhibitory Receptor Bioassays:

## Ordering information

| Co-Inhibitory Receptor Bioassays                      | Product Category | Cat. No.   | Components   | Assays in 96-well format |
|---|------------------|------------|--|--------------------------|
| <b>PD-1/PD-L1 Blockade Bioassay</b>                   |                  |            |  |                          |
| PD-1/PD-L1 Blockade Bioassay                          | Catalog          | J1250      | 1 x 1 vial PD-1 Effector Cells<br>1 x 1 vial PD-L1 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x Ham's F12 Medium<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System                | 120                      |
| PD-1/PD-L1 Blockade Bioassay 5X                       | Catalog          | J1255      | 5 x 1 vial PD-1 Effector Cells 5X<br>5 x 1 vial PD-L1 aAPC/CHO-K1 Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x Ham's F12 Medium<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System             | 600                      |
| PD-1/PD-L1 Blockade Bioassay, Propagation Model       | Catalog          | J1252      | 2 x 1 vial PD-1 Effector Cells<br>2 x 1 vial PD-L1 aAPC/CHO-K1 Cells   |                          |
| Control Ab (Anti-PD-1)                                | Catalog          | J1201      | 1 x 100 µg Control Ab (Anti-PD-1)  |                          |
| PD-L1 Negative Cells                                  | Catalog          | J1191      | 1 x 1 vial aAPC/CHO-K1 Cells   | 120                      |
| PD-L1 Negative Cells 5X                               | Catalog          | J1195      | 5 x 1 vial aAPC/CHO-K1 Cells   | 600                      |
| PD-L1 Negative Cells, Propagation Model               | CAS              | CS187110   | 1 x 1 vial aAPC/CHO-K1 Cells   |                          |
| <b>PD-1/PD-L2 Blockade Bioassay</b>                   |                  |            |  |                          |
| PD-1/PD-L2 Blockade Bioassay Kit                      | CAS              | CS187131-1 | 1 x 1 vial PD-1 Effector Cells<br>1 x 1 vial PD-L2 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x Bio-Glo™ Luciferase System<br>1 x 36 ml RPMI Medium<br>1 x Ham's F12 Medium                           | 120                      |
| PD-1/PD-L2 Blockade Bioassay Kit 5X                   | CAS              | CS187135-1 | 5 x 1 vial PD-1 Effector Cells<br>5 x 1 vial PD-L2 aAPC/CHO-K1 Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x Bio-Glo™ Luciferase System<br>5 x 36 ml RPMI Medium<br>5 x Ham's F12 Medium                           | 600                      |
| PD-1/PD-L2 Blockade Bioassay, Propagation Model       | CAS              | CS187130   | 2 x 1 vial PD-1 Effector Cells<br>2 x 1 vial PD-L2 Effector Cells aAPC/CHO-K1 Cells  |                          |
| <b>Mouse PD-1/PD-L1 Blockade Bioassay</b>             |                  |            |  |                          |
| Mouse PD-1/PD-L1 Blockade Bioassay                    | CAS              | CS303201   | 1 x 1 vial Mouse PD-1 Effector Cells<br>1 x 1 vial Mouse PD-L1 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x Ham's F12 Medium<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System    | 120                      |
| Mouse PD-1/PD-L1 Blockade Bioassay 5X                 | CAS              | CS303205   | 5 x 1 vial Mouse PD-1 Effector Cells 5X<br>5 x 1 vial Mouse PD-L1 aAPC/CHO-K1 Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x Ham's F12 Medium<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| Mouse PD-1/PD-L1 Blockade Bioassay, Propagation Model | CAS              | TBD        | 2 x 1 vial Mouse PD-1 Effector Cells<br>2 x 1 vial Mouse PD-L1 aAPC/CHO-K1 Cells   |                          |

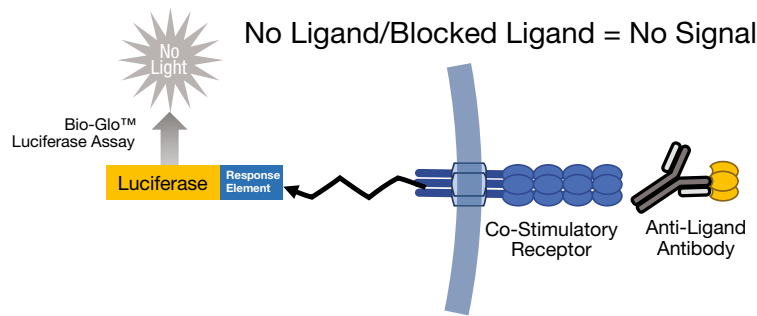
| Co-Inhibitory Receptor Bioassays                 | Product Category | Cat. No. | Components  | Assays in 96-well format |
|--|------------------|----------|---|--------------------------|
| <b>CTLA-4 Blockade Bioassay</b>                  |                  |          |   |                          |
| CTLA-4 Blockade Bioassay Kit                     | CAS              | JA3001   | CTLA-4 Effector Cells and aAPC/Raji Cells<br>1 x 1 vial CTLA-4 Effector Cells<br>1 x 1 vial aAPC/Raji Cells<br>4 ml Fetal Bovine Serum<br>1 x 36ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System                       | 120                      |
| CTLA-4 Blockade Bioassay Kit 5X                  | CAS              | JA3005   | CTLA-4 Effector Cells and aAPC/Raji Cells<br>5 x 1 vial CTLA-4 Effector Cells<br>5 x 1 vial aAPC/Raji Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System                   | 600                      |
| CTLA-4 Blockade Bioassay, Propagation Model      | CAS              | JA1400   | CTLA-4 Effector Cells<br>aAPC/Raji Cells  |                          |
| Control Ab (Anti-CTLA-4)                         | CAS              | JA1020   | 1 x 20 µg Control Ab (Anti-CTLA-4)  |                          |
| <b>LAG-3 Blockade Bioassay</b>                   |                  |          |   |                          |
| LAG-3 Blockade Bioassay Kit                      | Catalog          | JA1111   | 1 x 1 vial LAG-3 Effector Cells<br>1 x 1 vial MHCII APC Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 60 ml DMEM Medium<br>1 x 36 ml RPMI 1640 Medium<br>1 x 0.06 ml TCR Activating Antigen<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| LAG-3 Blockade Bioassay Kit 5X                   | Catalog          | JA1115   | 5 x 1 vial LAG-3 Effector Cells<br>5 x 1 vial MHCII APC Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 60 ml DMEM Medium<br>5 x 36 ml RPMI 1640 Medium<br>5 x 0.06 ml TCR Activating Antigen<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| LAG-3 Blockade Bioassay, Propagation Model       | Catalog          | JA1112   | 2 x 1 vial LAG-3 Effector Cells<br>2 x 1 vial MHCII APC Cells<br>1 x 0.5 ml TCR Activating Antigen  |                          |
| TCR Activating Antigen                           | Catalog          | K1201    | 1 x 0.5 ml TCR Activating Antigen   |                          |
| Control Ab (Anti-LAG-3)                          | Catalog          | K1150    | 1 x 100 µg Control Ab (Anti-LAG-3)  |                          |
| <b>TIGIT/CD155 Blockade Bioassay</b>             |                  |          |   |                          |
| TIGIT/CD155 Blockade Bioassay Kit                | Catalog          | J2201    | 1 x 1 vial TIGIT Effector Cells<br>1 x 1 vial CD155 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System  | 120                      |
| TIGIT/CD155 Blockade Bioassay Kit 5X             | Catalog          | J2205    | 5 x 1 vial TIGIT Effector Cells<br>5 x 1 vial CD155 aAPC/CHO-K1 Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System  | 600                      |
| TIGIT/CD155 Blockade Bioassay, Propagation Model | Catalog          | J2092    | 2 x 1 vial TIGIT Effector Cells<br>2 x 1 vial CD155 aAPC/CHO-K1 Cells   |                          |
| Control Ab (Anti-TIGIT)                          | Catalog          | J2051    | 1 x 100 µg Control Ab (Anti-TIGIT)  |                          |
| TIGIT Negative Cells ("thaw-and-use")            | Catalog          | J1921    | 1 x 1 vial TIGIT Negative Cells   |                          |

| Co-Inhibitory Receptor Bioassays                         | Product Category | Cat. No.  | Components  | Assays in 96-well format |
|--|------------------|-----------|---|--------------------------|
| <b>CD226/CD155 Blockade Bioassay</b>                     |                  |           |   |                          |
| CD226/CD155 Blockade Bioassay Kit                        | CAS              | CS1978F01 | 1 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>1 x 1 vial CD155 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| CD226/CD155 Blockade Bioassay, Propagation Model         | CAS              | TBD       | 2 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>2 x 1 vial CD155 aAPC/CHO-K1 Cells  |                          |
| <b>TIM-3 Bioassay<br/>(utilizes NanoLuc® Luciferase)</b> |                  |           |   |                          |
| TIM-3 Bioassay Kit                                       | Catalog          | JA2211    | 1 x 1 vial TIM-3 Effector Cells<br>1 x 1 vial TIM-3 Target Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo-NL™ Luciferase Assay System            | 120                      |
| TIM-3 Bioassay Kit 5X                                    | Catalog          | JA2215    | 5 x 1 vial TIM-3 Effector Cells<br>5 x 1 vial TIM-3 Target Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo-NL™ Luciferase Assay System            | 600                      |
| TIM-3 Bioassay, Propagation Model                        | Catalog          | JA2222    | 2 x 1 vial TIM-3 Effector Cells<br>2 x 1 vial TIM-3 Target Cells  |                          |
| Control Ab (Anti-TIM-3)                                  | Catalog          | K1210     | 1 x 100 µg Control Ab (Anti-TIM-3)  |                          |
| <b>BTLA/HVEM Blockade Bioassay</b>                       |                  |           |   |                          |
| BTLA/HVEM Blockade Bioassay Kit                          | CAS              | CS1978E04 | 1 x 1 vial BTLA Effector Cells<br>1 x 1 vial HVEM aAPC/CHO-K1<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System                  | 120                      |
| BTLA/HVEM Blockade Bioassay Kit 5X                       | CAS              | CS1978E08 | 5 x 1 vial BTLA Effector Cells<br>5 x 1 vial HVEM aAPC/CHO-K1<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System                  | 600                      |
| BTLA/HVEM Blockade Bioassay, Propagation Model           | CAS              | CS1978E01 | 2 x 1 vial BTLA Effector Cells<br>2 x 1 vial HVEM aAPC/CHO-K1 Cells   |                          |

| Co-Inhibitory Receptor Bioassays   | Product Category | Cat. No.  | Components  | Assays in 96-well format |
|--|------------------|-----------|---|--------------------------|
| <b>ICOS Blockade Bioassay</b><br><i>(utilizes NanoLuc® Luciferase)</i><br><i>The ICOS Bioassay is available in both BLOCKADE and AGONIST formats.</i><br><i>The AGONIST products are listed in the 'Co-Stimulatory Bioassays' section.</i> |                  |           |   |                          |
| ICOS Blockade Bioassay Kit   | CAS              | CS1979D16 | 1 x 1 vial ICOS Effector Cells<br>1 x 1 vial ICOSL aAPC/CHO-K1<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36ml RPMI 1640 Medium<br>1 x 25ml Ham's F12 Medium<br>1 x Bio-Glo-NL™ Luciferase Assay System        | 120                      |
| ICOS Blockade Bioassay Kit 5X  | CAS              | CS1979D20 | 5 x 1 vial ICOS Effector Cells<br>5 x 1 vial ICOSL aAPC/CHO-K1<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x 25 ml Ham's F12 Medium<br>5 x Bio-Glo-NL™ Luciferase Assay System      | 600                      |
| ICOS Blockade Bioassay, Propagation Model  | CAS              | CS1979D08 | 2 x 1 vial ICOS Effector Cells<br>2 x 1 vial ICOSL aAPC/CHO-K1 Cells  |                          |
| <b>CD28 Blockade Bioassay</b><br><i>The CD28 Bioassay is available in both BLOCKADE and AGONIST formats.</i><br><i>The AGONIST products are listed in the 'Co-Stimulatory Bioassays' section.</i>  |                  |           |   |                          |
| CD28 Blockade Bioassay Kit   | CAS              | CS1979C10 | 1 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>1 x 1 vial aAPC/Raji Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x 25 ml Ham's F12 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| CD28 Blockade Bioassay Kit 5X  | CAS              | CS1979C11 | 5 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>5 x 1 vial aAPC/Raji Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x 25 ml Ham's F12 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| CD28 Blockade Bioassay, Propagation Model  | CAS              | CS1979C09 | 2 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>2 x 1 vial aAPC/Raji Cells  |                          |
| <b>Bio-Glo™ Reagents</b>   |                  |           |   |                          |
| Bio-Glo™ Luciferase Assay System   | Catalog          | G7941     | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate  | 100                      |
| Bio-Glo™ Luciferase Assay System   | Catalog          | G7940     | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate   | 1000                     |
| Bio-Glo-NL™ Luciferase Assay System  | Catalog          | J3081     | 1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate  | 100                      |
| Bio-Glo-NL™ Luciferase Assay System  | Catalog          | J3082     | 1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate   | 1000                     |

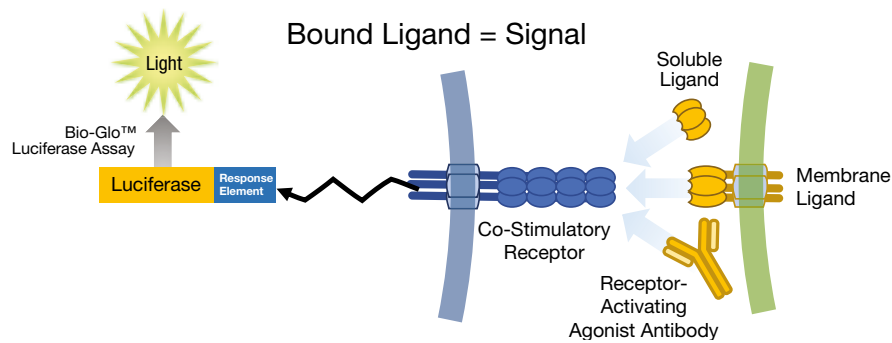
Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# Co-Stimulatory Receptor Bioassays



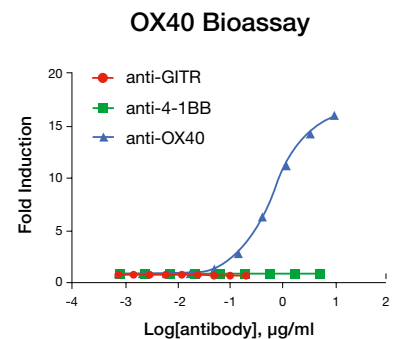
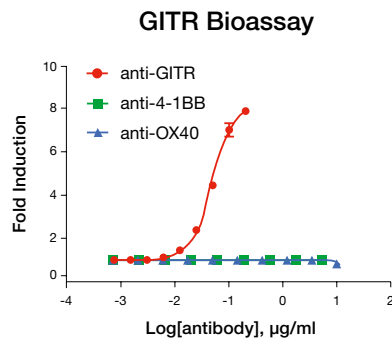
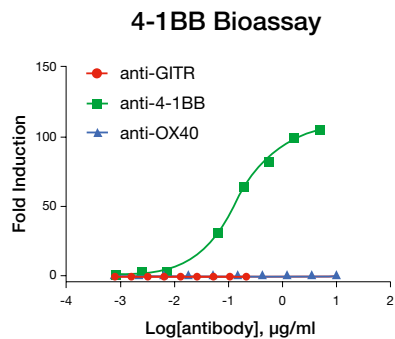
## Options:

GITR  
OX40  
4-1BB  
CD40  
ICOS  
CD28  
HVEM/LIGHT  
CD27  
DR3



**Co-stimulatory bioassay principle.** The bioassay typically consists of an effector cell that expresses the co-stimulatory receptor and a luciferase reporter gene driven by upstream activation of the co-stimulatory receptor.

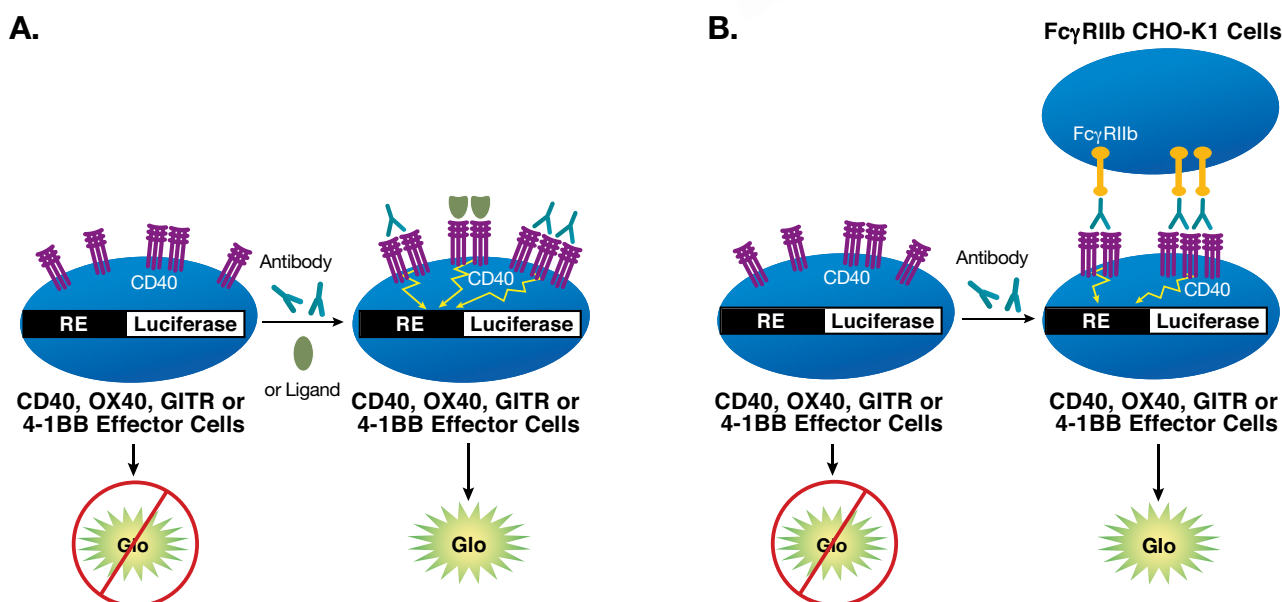
## Specificity of selected co-stimulatory Bioassays



**Antibody potency determination.** Bioassays for 4-1BB (left), GITR (center) and OX40 (right) receptors showed high sensitivity and specificity in measuring the potency of monoclonal antibodies against these co-stimulatory targets.



# OX40, CD40, GITR and 4-1BB Bioassays



**Representation of co-stimulatory bioassays (CD40, OX40, GITR or 4-1BB) with FcγRIIb-independent and FcγRIIb-dependent antibodies.** **A.** Assay with FcγRIIb-independent antibody. The bioassay consists of one engineered cell line: CD40, OX40, GITR or 4-1BB Effector Cells. In the absence of agonist antibody or ligand, the CD40, OX40, GITR or 4-1BB receptor is not activated and luminescence signal is low. The addition of agonist antibody or ligand induces the CD40, OX40, GITR or 4-1BB pathway-activated luminescence, which can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer. **B.** Assay with FcγRIIb-dependent antibody. The bioassay consists of two engineered cell lines: CD40, OX40, GITR or 4-1BB Effector Cells and FcγRIIb CHO-K1 Cells. In the presence of FcγRIIb CHO-K1 Cells, the anti-CD40, -OX40, -GITR or 4-1BB antibody is crosslinked, thereby inducing CD40, OX40, GITR or 4-1BB pathway activated luminescence, which can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer.

Some antibodies used to stimulate T cell activation via interaction with co-stimulatory receptors on the effector cells can be crosslinked via binding to FcγRIIb receptors on CHO-K1 cells to gain or increase assay response. Based on the properties of the antibody tested, the Co-Stimulatory Immune Checkpoint (i.e. OX40, CD40, GITR and 4-1BB) Bioassays can be conducted in a single genetically engineered cell system or dual genetically engineered cell systems. It is recommended to test the co-stimulatory bioassays with the FcγRIIb CHO-K1 cells when testing the antibody for the first time. If the antibody function is dependent upon FcγRIIb crosslinking, use the FcγRIIb CHO-K1 Cells every time.

The Co-stimulatory Effector Cells are provided in “thaw-and-use” formats as 1x and 5x kit sizes. FcγRIIb CHO-K1 Cells are available separately.

# Co-Stimulatory Receptor Bioassays:

## Ordering information

| Co-Stimulatory Receptor Bioassays | Product Category | Cat. No. | Components   | Assays in 96-well format |
|-----------------------------------|------------------|----------|--|--------------------------|
| <b>GITR Bioassay</b>              |                  |          |  |                          |
| GITR Bioassay Kit                 | Catalog          | JA2291   | 1 x 1 vial GITR Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System        | 120                      |
| GITR Bioassay Kit 5X              | Catalog          | JA2295   | 5 x 1 vial GITR Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System        | 600                      |
| GITR Bioassay, Propagation Model  | Catalog          | J2272    | 2 x 1 vial GITR Effector Cells   |                          |
| Control Ab (Anti-GITR)            | Catalog          | K1171    | Control Ab (Anti-GITR)   | 600                      |
| <b>4-1BB (CD137) Bioassay</b>     |                  |          |  |                          |
| 4-1BB Bioassay Kit                | Catalog          | JA2351   | 1 x 1 vial 4-1BB Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System       | 120                      |
| 4-1BB Bioassay Kit 5X             | Catalog          | JA2355   | 5 x 1 vial 4-1BB Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System       | 600                      |
| 4-1BB Bioassay, Propagation Model | Catalog          | J2332    | 2 x 1 vial 4-1BB Effector Cells  |                          |
| Control Ab (Anti-4-1BB)           | Catalog          | K1161    | 1 x 50 µg Control Ab (Anti-4-1BB)  | 600                      |
| <b>OX40 Bioassay</b>              |                  |          |  |                          |
| OX40 Bioassay Kit                 | Catalog          | JA2191   | 1 x 1 vial OX40 Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System        | 120                      |
| OX40 Bioassay Kit 5X              | Catalog          | JA2195   | 5 x 1 vial OX40 Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System        | 600                      |
| OX40 Bioassay, Propagation Model  | Catalog          | J2172    | 2 x 1 vial OX40 Effector Cells   |                          |
| Control Ab (Anti-OX40)            | Catalog          | K1191    | Control Ab (Anti-OX40)   | 600                      |
| <b>CD40 Bioassay</b>              |                  |          |  |                          |
| CD40 Bioassay Kit                 | Catalog          | JA2151   | 1 x 1 vial CD40 Effector Cells (U2OS)<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| CD40 Bioassay Kit 5X              | Catalog          | JA2155   | 5 x 1 vial CD40 Effector Cells (U2OS)<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| CD40 Bioassay, Propagation Model  | Catalog          | J2132    | 2 x 1 vial CD40 Bioassay Cells (U2OS)  |                          |
| Control Ab (Anti-CD40)            | Catalog          | K1181    | 1 x 50 µg Control Ab (Anti-CD40)   |                          |

| Co-Stimulatory Receptor Bioassays  | Product Category | Cat. No. | Components  | Assays in 96-well format |
|--|------------------|----------|---|--------------------------|
| <b>FcγRIIb/CHO-K1 Cells</b><br><i>Optimized for use in combination with the GLTR, 4-1BB, OX40, and CD40 Effector Cells</i> |                  |          |   |                          |
| FcγRIIb/CHO-K1 Cell Kit  | Catalog          | JA2251   | 1 x 1 vial FcγRIIb/CHO-K1 Cells<br>1 x 36 ml RPMI 1640 Medium | 120                      |
| FcγRIIb/CHO-K1 Cells Kit 5X  | Catalog          | JA2255   | 5 x 1 vial FcγRIIb/CHO-K1 Cells<br>5 x 36 ml RPMI 1640 Medium | 600                      |
| FcγRIIb/CHO-K1 Cell Line, Propagation Model  | Catalog          | J2232    | 2 x 1 vial FcγRIIb/CHO-K1 Cells                               |                          |

| HVEM/LIGHT Bioassay                       |     |           |   |     |
|---|-----|-----------|---|-----|
| HVEM/LIGHT Bioassay Kit                   | CAS | CS1979A30 | 1 x 1 vial HVEM Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| HVEM/LIGHT Bioassay Kit 5X                | CAS | CS1979A32 | 5 x 1 vial HVEM Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| HVEM/LIGHT Bioassay, Propagation Model    | CAS | CS1979A16 | 2 x 1 vial HVEM Effector Cells  |     |
| LIGHT CHO-K1 Cell Line, Propagation Model | CAS | TBD       | 2 x 1 vial LIGHT CHO-K1 Cells   |     |

| CD27 Bioassay                            |     |           |   |     |
|--|-----|-----------|---|-----|
| CD27 Bioassay Kit                        | CAS | CS1979A23 | 1 x 1 vial CD27 Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| CD27 Bioassay Kit 5X                     | CAS | CS1979A25 | 5 x 1 vial CD27 Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| CD70 CHO-K1 Cells ("thaw-and-use")       | CAS | CS1979A19 | 1 x 1 vial CD70 CHO-K1 Cells  | 120 |
| CD27 Bioassay, Propagation Model         | CAS | CS1979A15 | 2 x 1 vial CD27 Effector Cells  |     |
| CD70 CHO-K1 Cell Line, Propagation Model | CAS | CS1979A18 | 2 x 1 vial CD70 CHO-K1 Cells  |     |

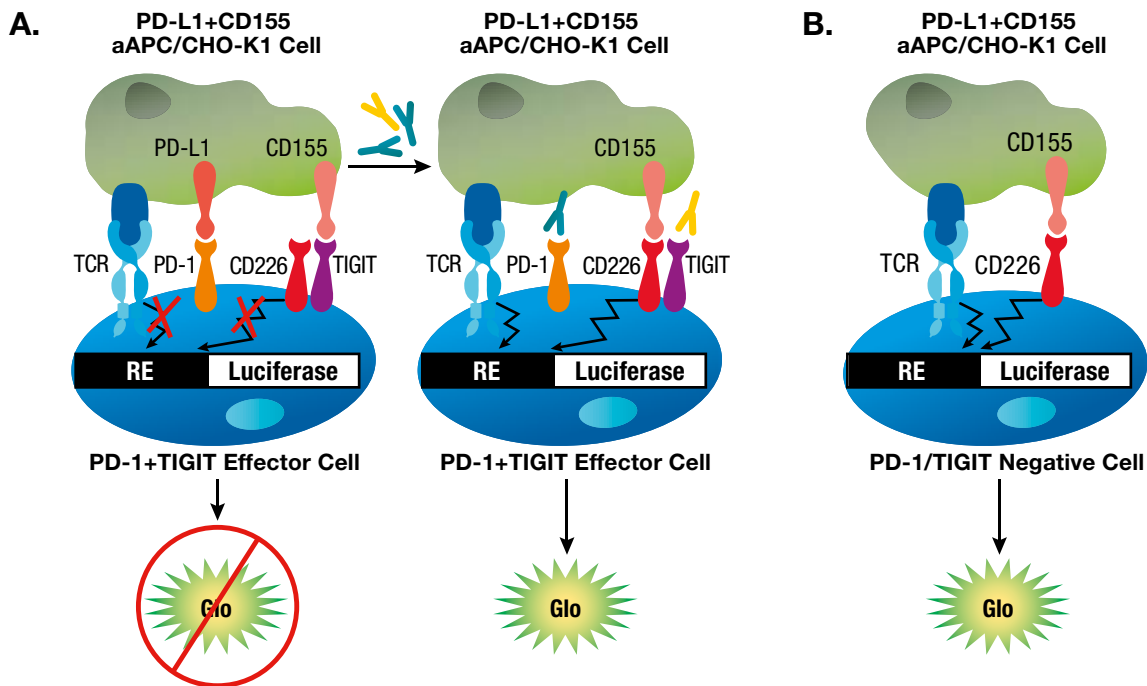
| Co-Stimulatory Receptor Bioassays                                       | Product Category | Cat. No.                         | Components  | Assays in 96-well format |
|---|------------------|----------------------------------|---|--------------------------|
| <b>DR3 Bioassay</b>   |                  |                                  |   |                          |
| DR3 Bioassay Kit  | CAS              | CS1979E03                        | 1 x 1 vial DR3 Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System  | 120                      |
| DR3 Bioassay Kit 5X   | CAS              | CS1979E06                        | 5 x 1 vial DR3 Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System  | 600                      |
| DR3 Bioassay, Propagation Model   | CAS              | CS1979E02                        | 2 x 1 vial DR3 Effector Cells   |                          |
| <b>ICOS Bioassay (Agonist format)</b><br>(utilizes NanoLuc® Luciferase) |                  |                                  |   |                          |
| ICOS Bioassay, Core Kit   | CAS              | CS1979D15                        | 1 x 1 vial ICOS Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36ml RPMI 1640 Medium<br>1 x Bio-Glo-NL™ Luciferase Assay System   | 120                      |
| ICOS Bioassay, Core Kit 5X  | CAS              | CS1979D18                        | 5 x 1 vial ICOS Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo-NL™ Luciferase Assay System  | 600                      |
| ICOS Bioassay, Propagation Model  | CAS              | Multiple configurations possible | 2 x 1 vial ICOS Effector Cells (CS1979D02)<br>+<br>2 x 1 vial FcγRIIb aAPC/CHO-K1 Cells (CS1979D04)<br><b>OR</b><br>2 x 1 vial aAPC/CHO-K1 Cells (CS187110)                         |                          |
| <b>CD28 Bioassay (Agonist format)</b>                                   |                  |                                  |   |                          |
| CD28 Bioassay, Core Kit   | CAS              | CS1979C06                        | 1 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x 25 ml Ham's F12 Medium<br>1 x Bio-Glo™ Luciferase Assay System         | 120                      |
| CD28 Bioassay, Core Kit 5X  | CAS              | CS1979C08                        | 5 x 1 vial TCR/CD3 Effector Cells (IL-2)<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>1 x 25 ml Ham's F12 Medium<br>1 x Bio-Glo™ Luciferase Assay System         | 600                      |
| CD28 Bioassay, Propagation Model  | CAS              | Multiple configurations possible | 2 x 1 vial TCR/CD3 Effector Cells (IL-2) (CPM) (J1631)<br>+<br>2 x 1 vial FcγRIIb aAPC/CHO-K1 Cells (CPM) (CS1979D04)<br><b>OR</b><br>2 x 1 vial aAPC/CHO-K1 Cells (CPM) (CS187110) |                          |

| Co-Stimulatory Receptor Bioassays  | Product Category | Cat. No.  | Components  | Assays in 96-well format |
|--|------------------|-----------|---|--------------------------|
| <b>FcγRIIb aAPC/CHO-K1 Cells</b><br><i>Optimized for use in combination with Effector Cells in the ICOS and CD28 bioassays for direct agonist measurement</i>                                |                  |           |   |                          |
| FcγRIIb aAPC/CHO-K1 Cells  | CAS              | CS1979D14 | 1 x 1 vial FcγRIIb aAPC/CHO-K1 Cells  | 120                      |
| FcγRIIb aAPC/CHO-K1 Cells 5x   | CAS              | CS1979D17 | 5 x 1 vial FcγRIIb aAPC/CHO-K1 Cells  | 600                      |
| FcγRIIb aAPC/CHO-K1 Cell Line, Propagation Model   | CAS              | CS1979D04 | 2 x 1 vial FcγRIIb aAPC/CHO-K1 Cells  |                          |
| <b>PD-L1 Negative Cells (aAPC/CHO-K1 Cells)</b><br><i>Optimized for use in combination with Effector Cells in the ICOS and CD28 bioassays for crosslinking-dependent agonist measurement</i> |                  |           |   |                          |
| PD-L1 Negative Cells (aAPC/CHO-K1 Cells)   | Catalog          | J1191     | 1 x 1 vial aAPC/CHO-K1 Cells  | 120                      |
| PD-L1 Negative Cells (aAPC/CHO-K1 Cells) 5X  | Catalog          | J1195     | 5 x 1 vial aAPC/CHO-K1 Cells  | 600                      |
| PD-L1 Negative Cells (aAPC/CHO-K1 Cells), Propagation Model  | CAS              | CS187110  | 2 x 1 vial aAPC/CHO-K1 Cells  |                          |
| <b>Bio-Glo™ Reagents</b>   |                  |           |   |                          |
| Bio-Glo™ Luciferase Assay System   | Catalog          | G7941     | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate        | 100                      |
| Bio-Glo™ Luciferase Assay System   | Catalog          | G7940     | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate       | 1000                     |
| Bio-Glo-NL™ Luciferase Assay System  | Catalog          | J3081     | 1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate  | 100                      |
| Bio-Glo-NL™ Luciferase Assay System  | Catalog          | J3082     | 1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate | 1000                     |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

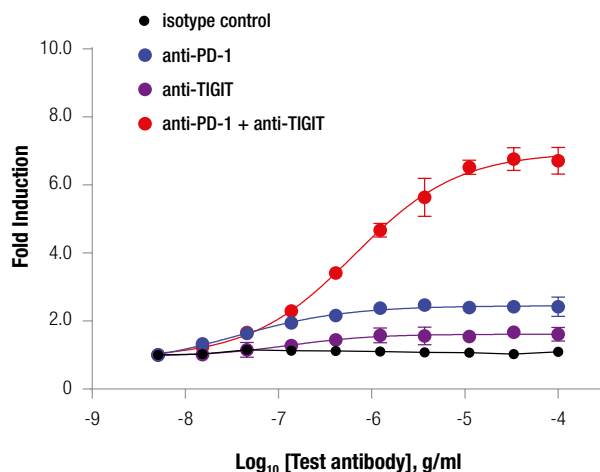
# Bioassays Combination Immunotherapy

## PD-1+TIGIT Combination Bioassay



**Representation of the PD-1+TIGIT Combination Bioassay.** The bioassay consists of two genetically engineered cell lines, PD-1+TIGIT Effector Cells and PD-L1+CD155 aAPC/CHO-K1 Cells. **A.** When co-cultured, PD-1 inhibits TCR pathway-activated luminescence, and TIGIT inhibits CD226 pathway-activated luminescence. The addition of anti-PD-1 Ab blocks PD-1 binding to PD-L1, resulting in full TCR pathway activation. The addition of anti-TIGIT Ab blocks the TIGIT/CD155 interaction, thereby re-establishing CD226 pathway-activated luminescence. Blocking of PD-1/PD-L1 and TIGIT/CD155 can be detected in a dose-dependent manner by addition of Bio-Glo™ Reagent and quantitation with a luminometer. **B.** When co-cultured with Effector Cells that do not express PD-1 or TIGIT (Negative Cells), TCR activation and CD226/CD155 induce luminescence.

### Synergy of the PD-1+TIGIT Combination Bioassay



The PD-1+TIGIT combination bioassay showed a twofold, dose-dependent increase in luciferase activity in response to either nivolumab or an anti-TIGIT antibody. Addition of a 1:1 ratio of anti-PD-1 and anti-TIGIT antibodies resulted in an eightfold increase in luciferase activity. Therefore, the bioluminescent reporter-based PD-1+TIGIT combination bioassay provides a quantitative measure of the synergetic effect of anti-PD-1 and anti-TIGIT immune checkpoint blocking antibodies on T cell activation.

# Combination Bioassays: Ordering information

| PD-1+TIGIT Combination Bioassay                   | Product Category | Cat. No. | Components  | Assays in 96-well format |
|---|------------------|----------|---|--------------------------|
| PD-1+TIGIT Combination Bioassay Kit               | Catalog          | J2211    | 1 x 1 vial PD-1+TIGIT Effector Cells<br>1 x 1 vial PD-L1+CD155 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x Ham's F12 Medium<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| PD-1+TIGIT Combination Bioassay Kit 5X            | Catalog          | J2215    | 5 x 1 vial PD-1+TIGIT Effector Cells<br>5 x 1 vial PD-L1+CD155 aAPC/CHO-K1 Cells<br>5 x 4 ml Fetal Bovine Serum<br>1 x Ham's F12 Medium<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| PD-1+TIGIT Combination Bioassay, Propagation Mode | Catalog          | J2102    | 2 x 1 vial PD-1+TIGIT Effector Cells<br>2 x 1 vial PD-L1+CD155 aAPC/CHO-K1 Cells  |                          |
| Control Ab (Anti-TIGIT)                           | Catalog          | J2051    | 1 x 100 µg Control Ab (Anti-TIGIT)  |                          |
| TIGIT Negative Cells ("thaw-and-use")             | Catalog          | J1921    | 1 x 1 vial TCR/CD3 Effector Cells (IL-2)  | 120                      |

| PD-1+LAG-3 Combination Bioassay                    |     |           |   |     |
|--|-----|-----------|---|-----|
| PD-1+LAG-3 Combination Bioassay Kit                | CAS | CS1978B11 | 1 x 1 vial PD-1+LAG-3 Effector Cells<br>1 x 1 vial PD-L1+MHCII APC Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x 36 ml DMEM Medium<br>1 x 0.006 mg TCR Activating Antigen<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| PD-1+LAG-3 Combination Bioassay Kit 5X             | CAS | CS1978B15 | 5 x 1 vial PD-1+LAG-3 Effector Cells<br>5 x 1 vial PD-L1+MHCII APC Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x 36 ml DMEM Medium<br>5 x 0.006 mg TCR Activating Antigen<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| PD-1+LAG-3 Combination Bioassay, Propagation Model | CAS | CS1978B07 | 2 x 1 vial PD-1+LAG-3 Effector Cells<br>2 x 1 vial PD-L1 Raji Cells   |     |

| PD-1+4-1BB Combination Bioassay<br>(utilizes NanoLuc® luciferase) |     |           |   |     |
|---|-----|-----------|---|-----|
| PD-1+4-1BB Combination Bioassay Kit                               | CAS | CS1978I05 | 1 x 1 vial PD-1+4-1BB Effector Cells<br>1 x 1 vial PD-L1 aAPC/CHO-K1 Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo-NL Luciferase Assay System | 120 |
| PD-1+4-1BB Combination Bioassay Kit 5X                            | CAS | CS1978I07 | 5 x 1 vial PD-1+4-1BB Effector Cells<br>5 x 1 vial PD-L1 aAPC/CHO-K1 Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo-NL Luciferase Assay System | 600 |
| PD-1+4-1BB Combination Bioassay, Propagation Model                | CAS | CS1978I03 | 2 x 1 vial PD-1+4-1BB Effector Cells<br>2 x 1 vial PD-L1 aAPC/CHO-K1 Cells  |     |



| <b>PD-1+CTLA-4 Combination Bioassay</b><br><i>(utilizes NanoLuc® luciferase)</i> | <b>Product Category</b> | <b>Cat. No.</b> | <b>Components</b>  | <b>Assays in 96-well format</b> |
|--|-------------------------|-----------------|--|---------------------------------|
| PD-1+CTLA-4 Combination Bioassay Kit   | CAS                     | CS1978D04       | 1 x 1 vial PD-1+CTLA-4 Effector Cells<br>1 x 1 vial PD-L1 aAPC/Raji Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo-NL Luciferase Assay System | 120                             |
| PD-1+CTLA-4 Combination Bioassay Kit 5X  | CAS                     | CS1978D08       | 5 x 1 vial PD-1+CTLA-4 Effector Cells<br>5 x 1 vial PD-L1 aAPC/Raji Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo-NL Luciferase Assay System | 600                             |
| PD-1+CTLA-4 Combination Bioassay, Propagation Model                              | CAS                     | CS1978D03       | 2 x 1 vial PD-1+CTLA-4 Effector Cells<br>2 x 1 vial PD-L1 aAPC/Raji Cells  |                                 |

| <b>Bio-Glo™ Reagents</b>            |         |       |   |      |
|-------------------------------------|---------|-------|---|------|
| Bio-Glo™ Luciferase Assay System    | Catalog | G7941 | 1 x 10 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate        | 100  |
| Bio-Glo™ Luciferase Assay System    | Catalog | G7940 | 1 x 100 ml Bio-Glo™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo™ Luciferase Assay Substrate       | 1000 |
| Bio-Glo-NL™ Luciferase Assay System | Catalog | J3081 | 1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate  | 100  |
| Bio-Glo-NL™ Luciferase Assay System | Catalog | J3082 | 1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate | 1000 |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# Cytokine and Growth Factor Bioassays

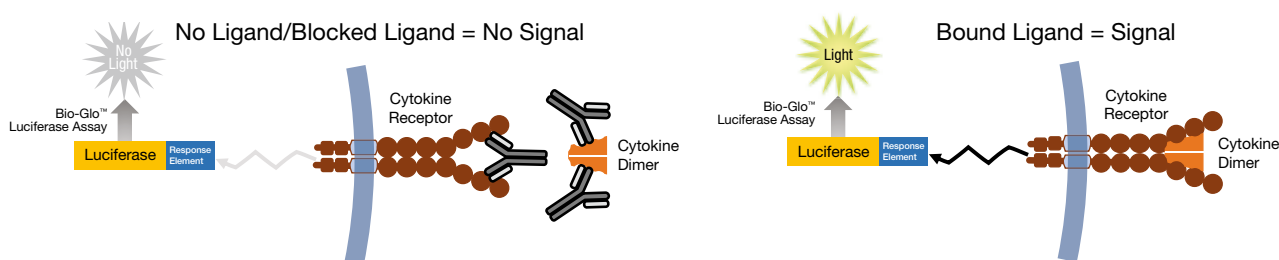
## Benefits

- ✓ Accelerate development of biosimilar drugs for cytokines and cytokine blockers
- ✓ Measure potency and manufacturing consistency of growth factor biosimilars and biobetters

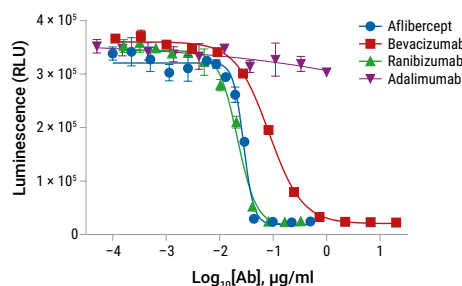
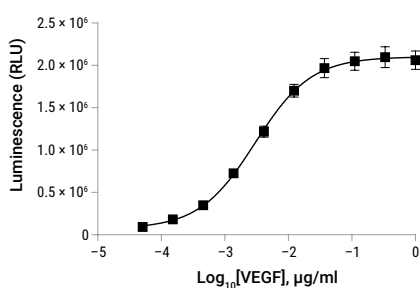
## Overview

Complex networks of cytokines and growth factors are involved in multiple cellular signaling pathways that are critical for cellular growth and differentiation. Alteration or disruption of these pathways by agents that modulate the binding of cytokines and growth factors to downstream receptors can lead to a variety of disease states, including immunosuppression, hepatotoxicity and cancer.

The Cytokine and Growth Factor Bioassays are luciferase reporter-based assays suitable for quantifying and monitoring the activity of ligands, as well as antibody-mediated blockade of ligand-receptor binding. These bioassays provide valuable tools for the development, stability testing and potency determination in the manufacture of cytokine and growth factor biosimilars and biobetters. Assays are available for VEGF, RANKL,  $\text{TNF}\alpha$ ,  $\text{TGF-}\beta$ ,  $\text{IFN}\beta$  and  $\text{IFN}\gamma$  and several interleukins, including IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-17, IL-22 and IL-23.



**Cytokine/growth factor bioassay principle.** Effector cells express a relevant cytokine or growth factor receptor and a luciferase reporter. Activation of the receptor by a ligand or agonist antibody leads to increased luciferase signal.



**VEGF Bioassay response.** Serial dilution of recombinant VEGF (left panel). The effect of various blockers of VEGF pathways was measured in the presence of an  $\text{EC}_{80}$  concentration of recombinant VEGF (right panel).

# Cytokine and Growth Factor Bioassays:

## Ordering information

| Cytokine & Growth Factor Bioassays   | Product Category | Cat. No.  | Components                   | Assays in 96-well format |
|--|------------------|-----------|------------------------------|--------------------------|
| <b>TNFα Bioassays</b>  |                  |           |                              |                          |
| TNFα Blockade Reporter Bioassay  | CAS              | CS177503  | TNFα Receptor Cells (HEK293) | 120                      |
| TNFα Reporter Cell Line  | Catalog          | E8520     | 2 x 1 vial NFκB HEK293 Cells |                          |
| TNFα Blockade Apoptosis Bioassay<br>Designed for use with Promega's Caspase-Glo 3/7 Assay Reagents (Cat#G8091)     | CAS              | CS1324A05 | TNFα Receptor Cells (U937)   | 120                      |
| Membrane TNFα Bioassay<br>Cross-listed in the Fc Effector chapter under anti-TNFα ADCC Bioassay                    | CAS              | CS185502  | Membrane TNFα Cells (CHO-K1) | 120                      |
| Membrane TNFα Bioassay, Propagation Model<br>Cross-listed in the Fc Effector chapter under anti-TNFα ADCC Bioassay | CAS              | CS185501  | Membrane TNFα Cells (CHO-K1) |                          |

|                                  |         |        |   |     |
|----------------------------------|---------|--------|---|-----|
| <b>VEGF Bioassay</b>             |         |        |   |     |
| VEGF Bioassay Kit                | Catalog | GA2001 | 1 x 1 vial KDR HEK293 Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 60 ml DMEM<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| VEGF Bioassay Kit, 5X            | Catalog | GA2005 | 5 x 1 vial KDR HEK293 Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 60 ml DMEM<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| VEGF Bioassay, Propagation Model | Catalog | GA1082 | 2 x 1 vial KDR Effector Cells   |     |
| Recombinant Human VEGF           | Catalog | J2371  | 1 x 1 vial Recombinant Human VEGF (10μg/50μl)   |     |

|                                   |     |           |  |     |
|-----------------------------------|-----|-----------|--|-----|
| <b>RANKL Bioassay</b>             |     |           |  |     |
| RANKL Bioassay Kit                | CAS | CS2018D03 | 2 x 1 vial RANK Effector Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System  | 120 |
| RANKL Bioassay Kit 5X             | CAS | CS2018D06 | 10 x 1 vial RANK Effector Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| RANKL Bioassay, Propagation Model | CAS | CS2018D01 | 2 x 1 vial RANK/NFκB U2OS Cells  |     |

|                                  |     |          |                             |  |
|----------------------------------|-----|----------|-----------------------------|--|
| <b>IL-1 Bioassay</b>             |     |          |                             |  |
| IL-1 Bioassay, Propagation Model | CAS | CS200401 | 2 x 1 vial hIL-8 A549 Cells |  |

|                                  |         |        |   |     |
|----------------------------------|---------|--------|---|-----|
| <b>IL-2 Bioassay</b>             |         |        |   |     |
| IL-2 Bioassay Kit                | Catalog | JA2201 | 1 x 1 vial IL-2 Bioassay Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120 |
| IL-2 Bioassay Kit 5X             | Catalog | JA2205 | 5 x 1 vial IL-2 Bioassay Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600 |
| IL-2 Bioassay, Propagation Model | Catalog | J2952  | 2 x 1 vial STAT5-RE CTLL-2 Cells  |     |

| Growth Factor & Cytokine Bioassays                      | Product Category | Cat. No.  | Components   | Assays in 96-well format |
|---|------------------|-----------|--|--------------------------|
| <b>IL-6 Bioassay</b>                                    |                  |           |  |                          |
| IL-6 Bioassay Kit                                       | Catalog          | JA2501    | 1 x 1 vial IL-6 Bioassay Cells including other components  | 120                      |
| IL-6 Bioassay Kit 5X                                    | Catalog          | JA2505    | 5 x 1 vial IL-6 Bioassay Cells including other components  | 600                      |
| IL-6 Bioassay, Propagation Model                        | Catalog          | J2992     | 2 x 1 vial IL-6 Bioassay Cells   |                          |
| <b>IL-12 Bioassay</b>                                   |                  |           |  |                          |
| IL-12 Bioassay Kit                                      | Catalog          | JA2601    | 1 x 1 vial IL-6 Bioassay Cells including other components  | 120                      |
| IL-12 Bioassay Kit 5X                                   | Catalog          | JA2605    | 5 x 1 vial IL-6 Bioassay Cells   | 600                      |
| IL-12 Bioassay, Propagation Model                       | Catalog          | J3042     | 2 x 1 vial IL-6 Bioassay Cells   |                          |
| <b>IL-15 Bioassay</b>                                   |                  |           |  |                          |
| IL-15 Bioassay Kit                                      | Catalog          | JA2011    | 1 x 1 vial IL-15 Bioassay Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x 36 ml RPMI 1640 Medium<br>1 x Bio-Glo™ Luciferase Assay System | 120                      |
| IL-15 Bioassay Kit 5X                                   | Catalog          | JA2015    | 5 x 1 vial IL-15 Bioassay Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x 36 ml RPMI 1640 Medium<br>5 x Bio-Glo™ Luciferase Assay System | 600                      |
| IL-15 Bioassay, Propagation Model                       | Catalog          | J2962     | 2 x 1 vial STAT5-RE CTLL-2 Cells   |                          |
| <b>IL-17 Bioassay</b><br>(utilizes NanoLuc® Luciferase) |                  |           |  |                          |
| IL-17 Bioassay Kit                                      | CAS              | CS2018C06 | 1 x 1 vial NFkB HeLa Cells including other components  | 120                      |
| IL-17 Bioassay Kit 5X                                   | CAS              | CS2018C08 | 5 x 1 vial NFkB HeLa Cells including other components  | 600                      |
| IL-17 Bioassay, Propagation Model                       | CAS              | CS2018C02 | 2 x 1 vial NFkB HeLa Cells   |                          |
| <b>IL-23 Bioassay</b>                                   |                  |           |  |                          |
| IL-23 Bioassay Kit                                      | Catalog          | JA2511    | 1 x 1 vial IL-23 Bioassay Cells including other components   | 120                      |
| IL-23 Bioassay Kit 5X                                   | Catalog          | JA2515    | 5 x 1 vial IL-23 Bioassay Cells including other components   | 600                      |
| IL-23 Bioassay, Propagation Model                       | Catalog          | J3002     | 2 x 1 vial IL-23 Bioassay Cells  |                          |

| Growth Factor & Cytokine Bioassays   | Product Category | Cat. No.  | Components  | Assays in 96-well format |
|--|------------------|-----------|---|--------------------------|
| <b>TGF-<math>\beta</math> Bioassay</b><br>(utilizes NanoLuc <sup>®</sup> Luciferase) |                  |           |   |                          |
| TGF- $\beta$ Bioassay Kit  | CAS              | CS2018F03 | 1 x 1 vial TGF- $\beta$ R Cells<br>1 x 4 ml Fetal Bovine Serum<br>1 x Ham's F12 Medium<br>1 x Bio-Glo-NL Luciferase Assay System  | 120                      |
| TGF- $\beta$ Bioassay Kit 5X   | CAS              | CS2018F06 | 5 x 1 vials TGF- $\beta$ R Cells<br>5 x 4 ml Fetal Bovine Serum<br>5 x Ham's F12 Medium<br>5 x Bio-Glo-NL Luciferase Assay System | 600                      |
| TGF- $\beta$ Bioassay, Propagation Model   | CAS              | CS2018F01 | 2 x 1 vials TGF- $\beta$ R Cells  |                          |
| <b>BCMA Bioassay</b><br>(utilizes NanoLuc <sup>®</sup> Luciferase)                   |                  |           |   |                          |
| BCMA Bioassay, Propagation Model   | CAS              | TBD       | 2 x 1 vials BCMA Cells (CPM)  |                          |
| <b>IFN<math>\alpha</math>/IFN<math>\beta</math> Reporter Cell Line</b>               |                  |           |   |                          |
| IFN $\alpha$ /IFN $\beta$ Reporter Cell Line   | CAS              | CS190701  | 2 x 1 vial ISRE HEK293 Cells  |                          |
| <b>IFN<math>\gamma</math> Reporter Cell Line</b>                                     |                  |           |   |                          |
| IFN $\gamma$ Reporter Cell line  | CAS              | TBD       | 2 x 1 vial GAS HeLa Cells   |                          |
| <b>EPO Reporter Cell Line</b>  |                  |           |   |                          |
| EPO Reporter Cell Line   | CAS              | CS204002  | 2 x 1 vial STAT5-RE TF-1 Cells  |                          |
| <b>GM-CSF Reporter Cell Line</b>   |                  |           |   |                          |
| GM-CSF Reporter Cell Line  | CAS              | CS204002  | 2 x 1 vial STAT5-RE TF-1 Cells  |                          |
| <b>EGF Reporter Cell Line</b>  |                  |           |   |                          |
| EGF Reporter Cell Line   | CAS              | CS181201  | 2 x 1 vial SRE HEK293 Cells   |                          |
| <b>G-CSF Reporter Cell line</b>  |                  |           |   |                          |
| G-CSF Reporter Cell Line   | CAS              | TBD       | 2 x 1 vial SIE U937 Cells   |                          |
| <b>Bio-Glo<sup>™</sup> Reagents</b>  |                  |           |   |                          |
| Bio-Glo <sup>™</sup> Luciferase Assay System   | Catalog          | G7941     | 1 x 10 ml Bio-Glo <sup>™</sup> Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo <sup>™</sup> Luciferase Assay Substrate              | 100                      |
| Bio-Glo <sup>™</sup> Luciferase Assay System   | Catalog          | G7940     | 1 x 100 ml Bio-Glo <sup>™</sup> Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo <sup>™</sup> Luciferase Assay Substrate             | 1000                     |
| Bio-Glo-NL <sup>™</sup> Luciferase Assay System                                      | Catalog          | J3081     | 1 x 10 ml Bio-Glo-NL <sup>™</sup> Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL <sup>™</sup> Luciferase Assay Substrate        | 100                      |
| Bio-Glo-NL <sup>™</sup> Luciferase Assay System                                      | Catalog          | J3082     | 1 x 100 ml Bio-Glo-NL <sup>™</sup> Luciferase Assay Buffer<br>1 x 1 vial Bio-Glo-NL <sup>™</sup> Luciferase Assay Substrate       | 1000                     |

Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

# Custom Assay Services

## Develop custom bioassays for your target of interest

- ✓ **Cell Engineering**  
Effector and target cell engineering to reflect biologically relevant drug MOA
- ✓ **Assay Development and Qualification**  
Optimization using DOE and qualification according to ICH guidelines
- ✓ **Cryopreserved “thaw-and-use” Cells**  
Large-scale manufacture of assay-ready bioassay cells

Promega has the tools, expert staff and state-of-the-art facilities to support complete custom solutions for biochemical and cell-based assays. We provide all the post-delivery support to ensure your assay works in your hands.

For general inquiries, e-mail:

[CAS@promega.com](mailto:CAS@promega.com)

For product information, visit:

[www.promega.com/cas](http://www.promega.com/cas)



To learn more about Promega bioassays for biologics, visit:

[www.promega.com/BetterBioassay](http://www.promega.com/BetterBioassay)

# GloMax® Navigator: Microplate Luminometer

## High-Performance, Easy-to-Use Detection System to Simplify Your Research

The GloMax® Navigator System is an easy-to-use microplate luminometer integrated with Promega chemistries for superior assay performance. The system provides researchers excellent luminescence sensitivity and dynamic range for both strong and weak bioluminescence experimental samples as well as seamless integration with Promega industry-leading bioluminescent reporter-based, cell-based and biochemical assays.

GloMax® Navigator is operated by an integrated Tablet PC which provides quick and easy navigation through the control options. Exporting your results is easy with a variety of options including exporting to your local data network, USB flash drive and cloud-based storage locations. The GloMax® Navigator software provides the technical elements of a FDA 21 CFR Part 11-compliant system (user authentication and authorization, data integrity and protection, electronic signatures and audit trails) when used with the appropriate laboratory workflow.

### Easy-to-use

Intuitive touchscreen display, preloaded protocols and automatic instrument gain adjustments

### Integrated with Promega assays

Preloaded Promega protocols and optimized instrument settings

### Superior performance

Broad dynamic range, low well-to-well cross talk and high sensitivity for detecting of low-level samples

### Service and support

Comprehensive one-year standard warranty and a full line of additional service products including Installation and Operation Qualification (IQ/OQ)

As well as the GloMax® Navigator System, Promega also offers Multimode reader systems with different configurations. These include high-performance luminescence, fluorescence, UV-Visible absorbance, BRET and FRET, two-color filtered luminescence and kinetic measurement capabilities.

**For further information, please visit:**  
[www.promega.com/glomax](http://www.promega.com/glomax)





# Automated Plasmid Purification for Biologics

## Plasmid purification for challenging applications

- **Automated High-Throughput Mini- and Midipreps**
- Centrifugation-free methods based on magnetic beads
- Rapid protocols **with or without endotoxin** removal
- **High quantity and purity** plasmids
- Compatible with almost all available liquid handlers
- Automation experts offering assistance and customized solutions



### Build your High-Throughput Solutions with Automation Experts

Developing an implementable solution for high-throughput nucleic acid purification can be challenging for a variety of reasons.

Promega's Field Support Scientists (FSS) together with our internal engineering and applications support teams work with you to develop a custom, optimized high-throughput solution to meet your specific needs. You will gain expert support either to optimize your current workflow or to help you to get started with automation.

### How we can support your lab needs:

- We consult with you to deeply understand your unique workflow and instrumentation requirements
- We assist you with implementation, optimization and/or automation of our technologies onsite in your lab
- We develop and provide customized training associated with new protocols to ensure your team is confident and qualified
- We consult and assist in improving laboratory efficiency and help with troubleshooting and with solving current issues

**For more information or to request a consult please contact us:**

**[HTgenomics@promega.com](mailto:HTgenomics@promega.com)**

**Or find your local Sales Support here:**

**[www.promega.com/support](http://www.promega.com/support)**

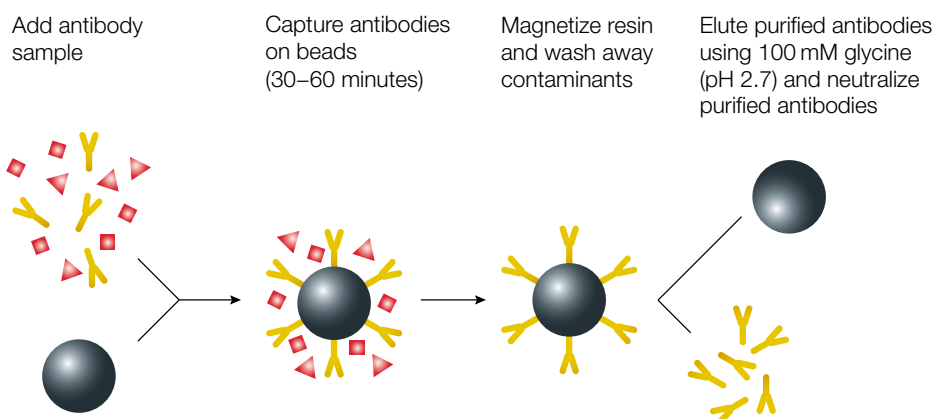
# Antibody Purification

## Manual or Automated Antibody Purification from Different Sample Types

Magne™ Protein A and Magne™ Protein G Beads are magnetic affinity beads with high specificity and improved capacity for binding antibodies from cell culture supernatant, ascites fluid and serum samples. Paramagnetic beads are composed of iron encapsulated in macroporous cellulose with low non-specific binding. A novel attachment chemistry of Protein A and Protein G allows for superior purification and recovery of concentrated antibodies from small input volumes (20 µl) by decreasing losses normally associated with handling of small volumes and nonmagnetic resins.

Magne™ Protein A and Magne™ Protein G Beads offer a convenient method for achieving high purity and recovery of monoclonal and polyclonal antibodies from biological samples. The superb magnetic properties allow rapid and efficient capture of antibodies either with manually processed samples or in a high-throughput manner using the Promega ReliaPrep™ LV 32 HSM Instrument or any other suitable robotic platform such as the Beckman Coulter Biomek® FX.

### Antibody purification using Magne™ Protein A Beads or Magne™ Protein G Beads



## High capacity and specificity

Magne™ Protein A and Magne™ Protein G Beads allow exceptional antibody yields (capacities in excess of 25 mg per 1 ml of beads depending on species and isotype) from diverse sample types such as serum, ascites and cell media. The binding selectivity for immunoglobulins prevents co-purification of albumin and other protein contaminants.

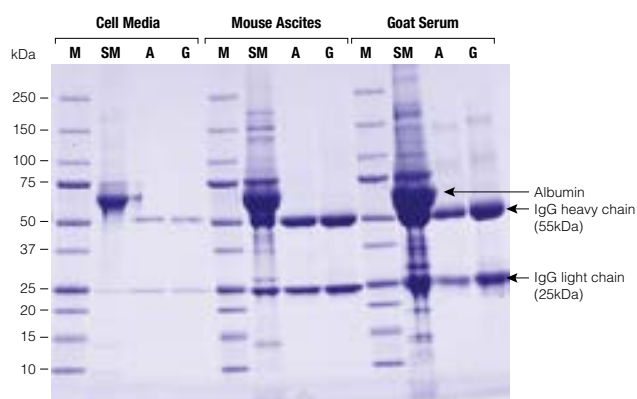
## Optimized performance

Robust magnetic response makes the Magne™ Protein A and Magne™ Protein G Beads ideal for high-throughput, automated applications. Validated protocols are available for microscale (20 µl) to medium-scale (50 ml) sample volumes.

## Efficient recovery

The magnetic method minimizes antibody losses encountered during column chromatography, dialysis and concentration steps found in traditional antibody purification protocols.

## Antibody purified from various sample types using the Magne™ Protein A and Magne™ Protein G Beads.



Antibody was purified from 50 µl of cell culture media (mouse IgG1), mouse ascites (IgG2a) and goat serum with 50 µl of Magne™ Protein A Beads (A) and Magne™ Protein G Beads (G), respectively, using published protocols (TM371).

Samples were separated via SDS-PAGE by adding 1 µl of starting material (SM) or 5 µl of purified sample (A or G) and stained with Coomassie®-based stain.

## Ordering information

| Product                                 | Size            | Cat. No. |
|---|-----------------|----------|
| Magne™ Protein G Beads, 20% Slurry      | 1 ml            | G7471    |
| Magne™ Protein G Beads, 20% Slurry      | 5 ml (5 × 1 ml) | G7472    |
| Magne™ Protein G Beads, 20% Slurry      | 50 ml           | G7473    |
| Magne™ Protein A Beads, 20% Slurry      | 1 ml            | G8781    |
| Magne™ Protein A Beads, 20% Slurry      | 5 ml (5 × 1 ml) | G8782    |
| Magne™ Protein A Beads, 20% Slurry      | 50 ml           | G8783    |
| HSM 2.0 Instrument Heater Shaker Magnet | 1 each          | A2715    |

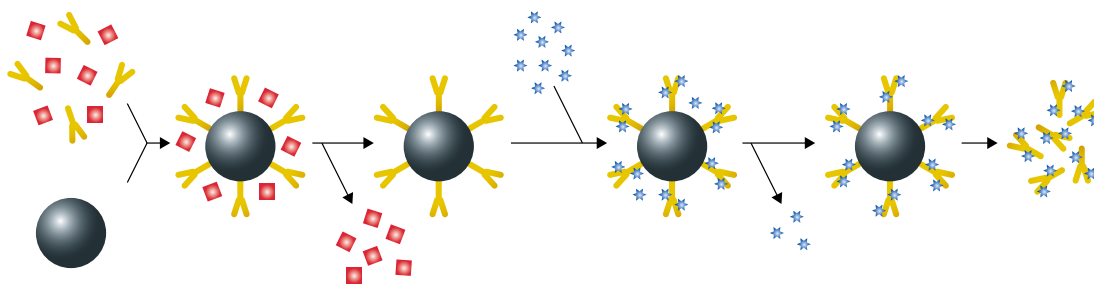
# On-Bead Antibody Conjugation

## On-Bead Antibody Conjugation using High Capacity Magnetic Protein A and Protein G Beads

Antibody conjugation is required for many different applications including the generation of Antibody Drug Conjugates (ADCs). To simplify the workflow for antibody conjugation and to increase throughput, high capacity Magne™ Protein A and Magne™ Protein G beads can be used for on-bead antibody conjugation. Antibodies are captured on-bead from cell media, serum or ascites without the need for pre-purification. Simple wash steps are performed to remove unreacted small molecules, avoiding the requirement for dialysis steps. Eluted antibodies are compatible with downstream applications such as cell internalization studies and ADCC assays.

### Schematic diagram of on-bead antibody conjugation using magnetic beads

|   |                           |  |  |  |
|---|---------------------------|--|--|--|
| Capture antibody with<br>Magne™ Protein A or<br>Protein G Beads | Wash away<br>contaminants | Buffer exchange.<br>Add reactive<br>labeling reagent | Wash away<br>unreacted<br>labeling reagent | Elute and neutralize<br>to obtain purified and<br>labeled antibodies |
|---|---------------------------|--|--|--|



### Advantages of on-bead antibody conjugation

- No pre-purification, dialysis and concentration steps required
- Highly concentrated labeled antibody
- Sample sizes from 20 µl to 50 ml
- Automatable for 1–96 samples

# pHAb Sensor Dye

## Set-up Homogeneous Plate-based Antibody-Internalization Assays using pHAb Sensor Fluorescent Dyes

A key requirement for candidate antibodies suitable for Antibody Drug Conjugate (ADC) applications is their ability to get internalized inside the cells. Promega's new pHAb Sensor Dyes in combination with on-bead conjugation facilitates screening of candidate antibodies for their internalization properties. The pHAb Sensor Dyes are non-fluorescent molecules at neutral pH but turns highly fluorescent in acidic environments (e.g. endosomal compartments). The excitation and emission maxima of antibodies labelled with pH sensor dye are 532 nm and 560 nm, respectively. Internalization of antibodies can be recorded with a plate-reader, FACS and (confocal) microscopy.

### Two types of pHAb Sensor Dyes are available:

1. pHAb amine reactive dye contains a succinimidyl ester (SE) reactive group, designed to label antibodies at primary amine of lysines.
2. pHAb thiol reactive dye contains a maleimide (ME) reactive group, designed to label antibodies at thiols from reduced cysteines in antibody hinge region.

### Features & benefits

- Streamlined protocol for antibody labeling and internalization assays
- Very bright label at low pH
- High solubility of labeled antibody
- No washing steps required after the addition to cells
- Allows real-time measurement using a plate reader

### Ordering information

| Product                 | Size    | Cat. No. |
|-------------------------|---------|----------|
| pHAb Amine Reactive Dye | 1×250µg | G9841    |
| pHAb Amine Reactive Dye | 4×250µg | G9845    |
| pHAb Thiol Reactive Dye | 1×250µg | G9831    |
| pHAb Thiol Reactive Dye | 4×250µg | G9835    |

Please contact Promega for additional information

# Magnetic Separation Devices

## Manual or automated antibody-capturing using magnetic beads

Promega offers a wide range of magnetic devices for separations from 0.5 ml microcentrifuge tubes to 15 ml or 50 ml conical tubes, to 96- and 384-well standard and deep-well plates. The magnetic separation device for plates is useful for both manual and automated liquid-handling.

### Ordering information

#### MagneSphere® Technology Magnetic Separation Stands



Two-position. Up to two sample volumes (50 µl–1.0 ml)  
Cat. No. Z5331, Z5332, Z5333



Twelve-position. Up to two sample volumes (50 µl–1.0 ml)  
Cat. No. Z5341, Z5342, Z5343



PolyAtract® System 1000 Magnetic Separation Stand. One sample volume (1–50 ml) Cat. No. Z5410

#### MagnaBot® Magnetic Separation Devices



MagnaBot® 96 Magnetic Separation Device for 96-well standard or deep well plates (20 µl–1.0 ml) Cat. No. V8151



MagnaBot® II Magnetic Separation Device for 96-well plate Cat. No. V8351



MagnaBot® 384 Magnetic Separation Device for 96-well plate Cat. No. V8241

# Reagents for Antibody Sample Preparation – IdeS/Z Proteases

## Antibody Fragmentation & Characterization

### IdeS Protease and IdeZ Protease

IdeS/IdeZ Proteases are engineered recombinant proteases that cleaves Immunoglobulin G (IgG) with high specificity at a single site below the hinge region, yielding F(ab')<sub>2</sub> and Fc fragments. These fragments are further reduced yielding three fragments of approximately 25 kDa (Fd', Fc/2 and LC) that are ideal for characterization by LC/MS. The smaller fragments facilitate accurate mass measurements that enable detection of posttranslational modifications (PTMs), such as glycoform profiles, C-terminal lysine variants, N-terminal pyroglutamate and oxidation.

### Features & benefits

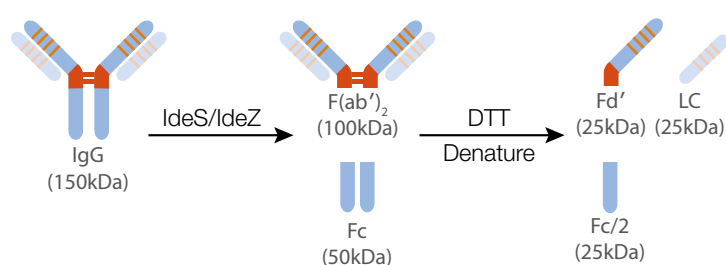
**Fast and easy:** Digestion in 30 minutes with no optimization

**Highly specific and reproducible:** Cleaves exclusively at a single site below the hinge to produce F(ab')<sub>2</sub> and Fc fragments

**High performance:** Essentially 100 % complete digestion, no-over digestion

**Cleavage specificity:** Both IdeS and IdeZ Proteases effectively cleave human IgG1, IgG2, IgG3 and IgG4, monkey, sheep, rabbit, humanized and chimeric IgGs as well as Fc-fusion proteins. However, mouse IgG2a and mouse IgG3 are cleaved by IdeZ Protease only

### Schematic showing cleavage specificity of IdeS and IdeZ Proteases



### Ordering information

| Product       | Size                             | Conc.       | Cat. No. |
|---------------|----------------------------------|-------------|----------|
| IdeS Protease | 5.000 units                      | lyophilized | V7511    |
| IdeS Protease | 25.000 units<br>(5 × 5000 units) | lyophilized | V7515    |
| IdeZ Protease | 5.000 units                      | lyophilized | V8341    |
| IdeZ Protease | 25.000 units<br>(5 × 5000 units) | lyophilized | V8345    |

For Research Use Only. Not for Use in Diagnostic Procedures

Promega is the first provider of proteases for sample preparation. Beside first class and consistent product quality, Promega offers in addition lot testing and lot reservation, on-time delivery, long-term delivery contracts as well as customer-specific packaging. Promega sites and departments are certified by ISO 9001:2015 and ISO 13485:2003, respectively.



# Reagents for Antibody Sample Preparation – AccuMAP™

## Suppression of Sample Preparation-induced Artefacts

### AccuMAP™ Low pH Protein Digestion Kit

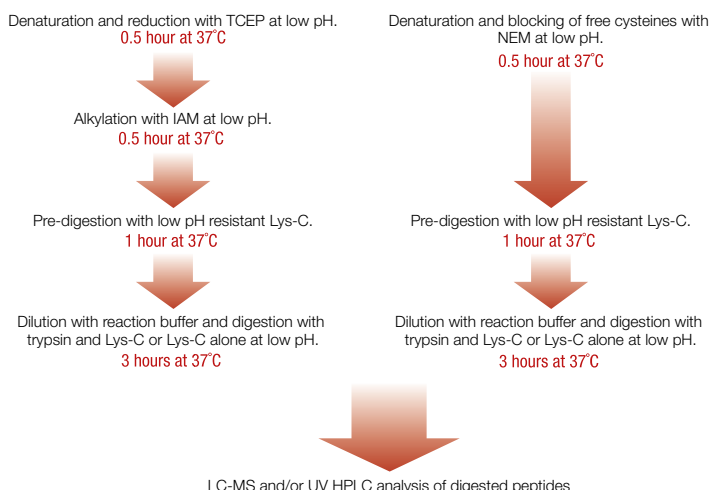
The AccuMAP™ Low pH Protein Digestion Kit is designed for accurate, reproducible characterization of proteins by peptide mapping using LC/MS and UV HPLC. The entire sample preparation is performed at low (mildly acidic) pH to suppress artificial deamidation and disulfide bond scrambling. The kit also contains an optional agent for suppression of protein oxidation during sample preparation.

#### Features & benefits

- Suppression of sample preparation-induced artificial deamidation, disulfide bond scrambling and oxidation over the course of sample preparation. Pre-existing non-enzymatic modifications remain intact
- Efficient reduction, alkylation and digestion at low pH
- High reproducibility

### Schematic diagram of sample preparation by digestion performed with the AccuMAP™ Low pH Protein Digestion Kit

#### A. Low pH digestion under reducing conditions    B. Low pH digestion under non-reducing conditions



#### Ordering information

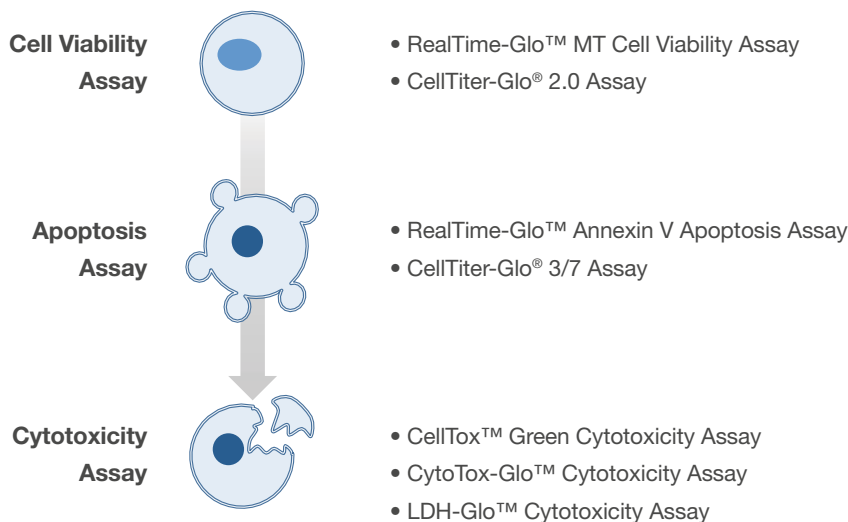
| Product                                      | Size                          | Cat. No. |
|--|-------------------------------|----------|
| AccuMAP™ Low pH Protein Digestion Kit*, Mini | Sufficient for 500 µg protein | VA1040   |
| AccuMAP™ Low pH Protein Digestion Kit*, Maxi | Sufficient for 5 mg protein   | VA1050   |

\* Kit components: Denaturing Solution (contains GuHCl), Low pH Reaction Buffer, TCEP, NEM, IAM, Oxidation Suppressant Solution, Modified Trypsin, Low pH Resistant Lys-C.

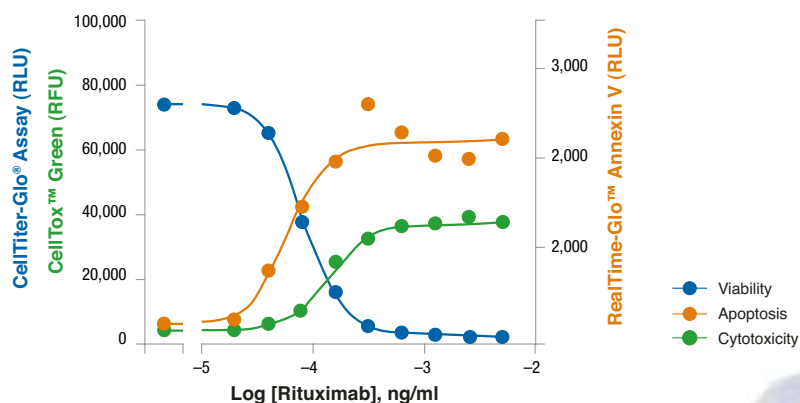


## Bioluminescent Cell-Based Assays to Study Cell Health and Cellular Responses

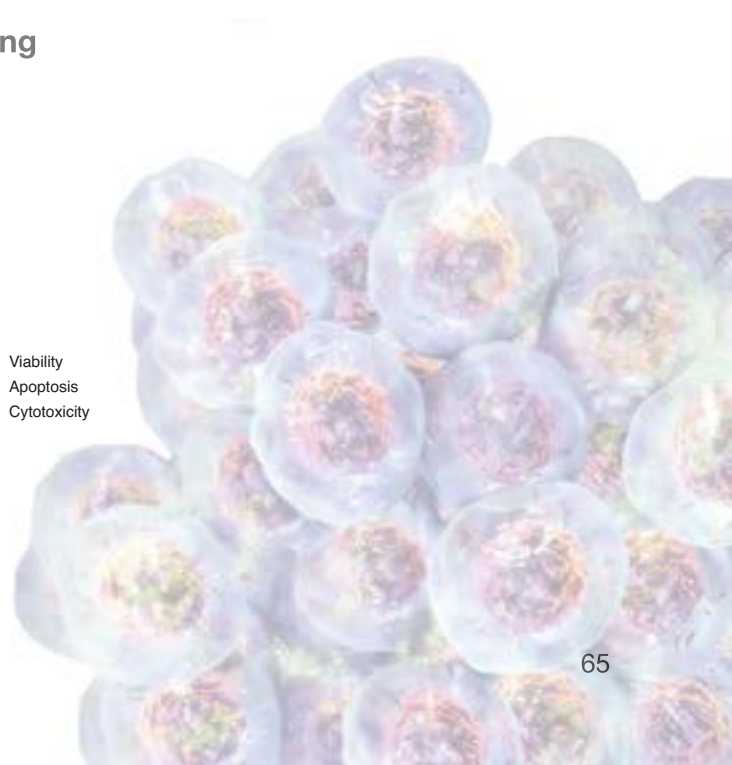
The assessment of cell health and cellular responses after experimental manipulation continue to be a very important aspect of experimental biology. Promega offers a wide selection of sensitive and easy to-use cell-based assay formats to interrogate these aspects of cellular biology including our newest real-time and 3D cultures assays. In addition, combinations of these assays in a multiplexing approach is possible to determine several parameters simultaneously facilitating the interpretation of results and improve data quality.



### Determine cell death mechanism by multiplexing



Learn more about Cell Health Assays  
[www.promega.com/cellhealth](http://www.promega.com/cellhealth)



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