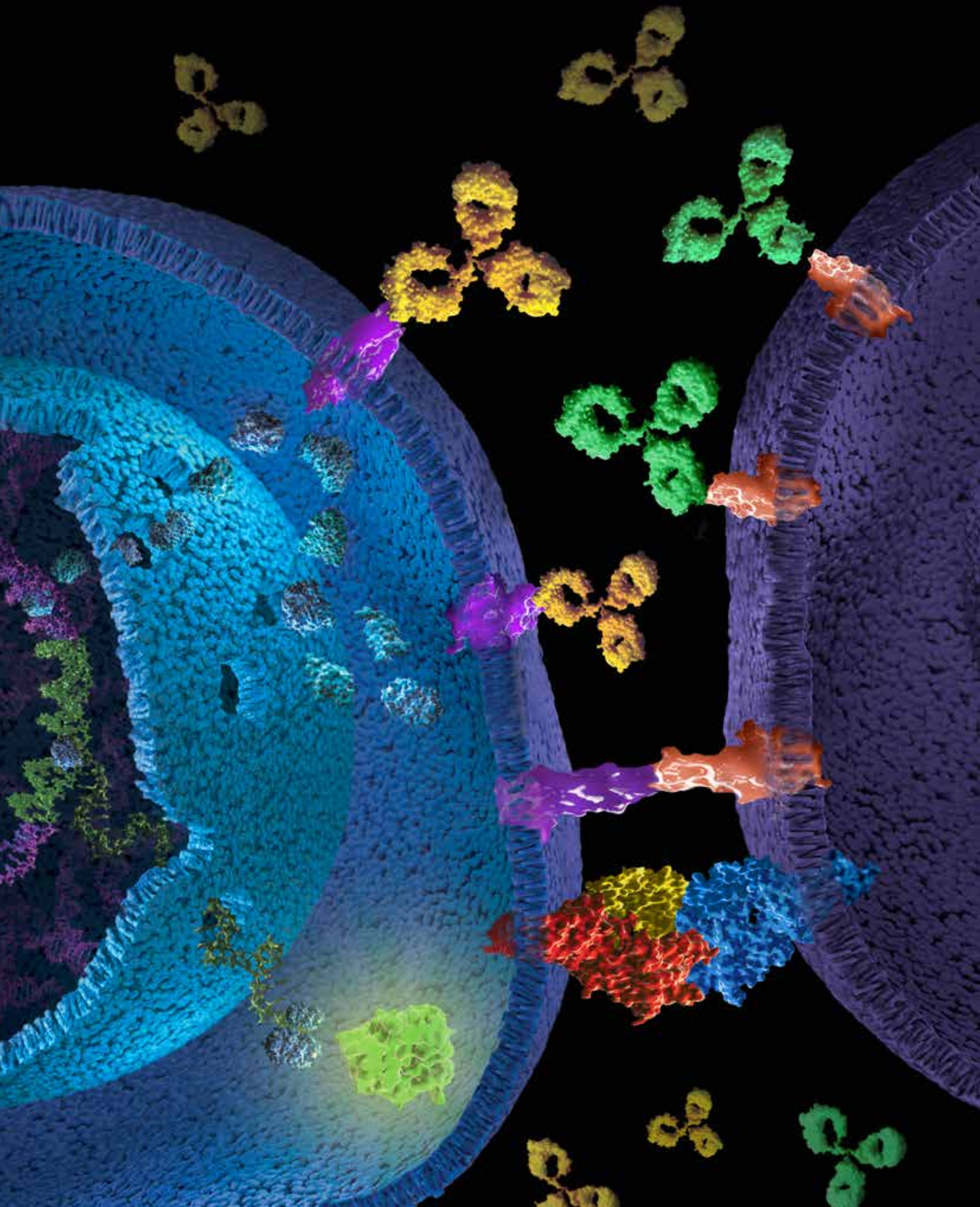
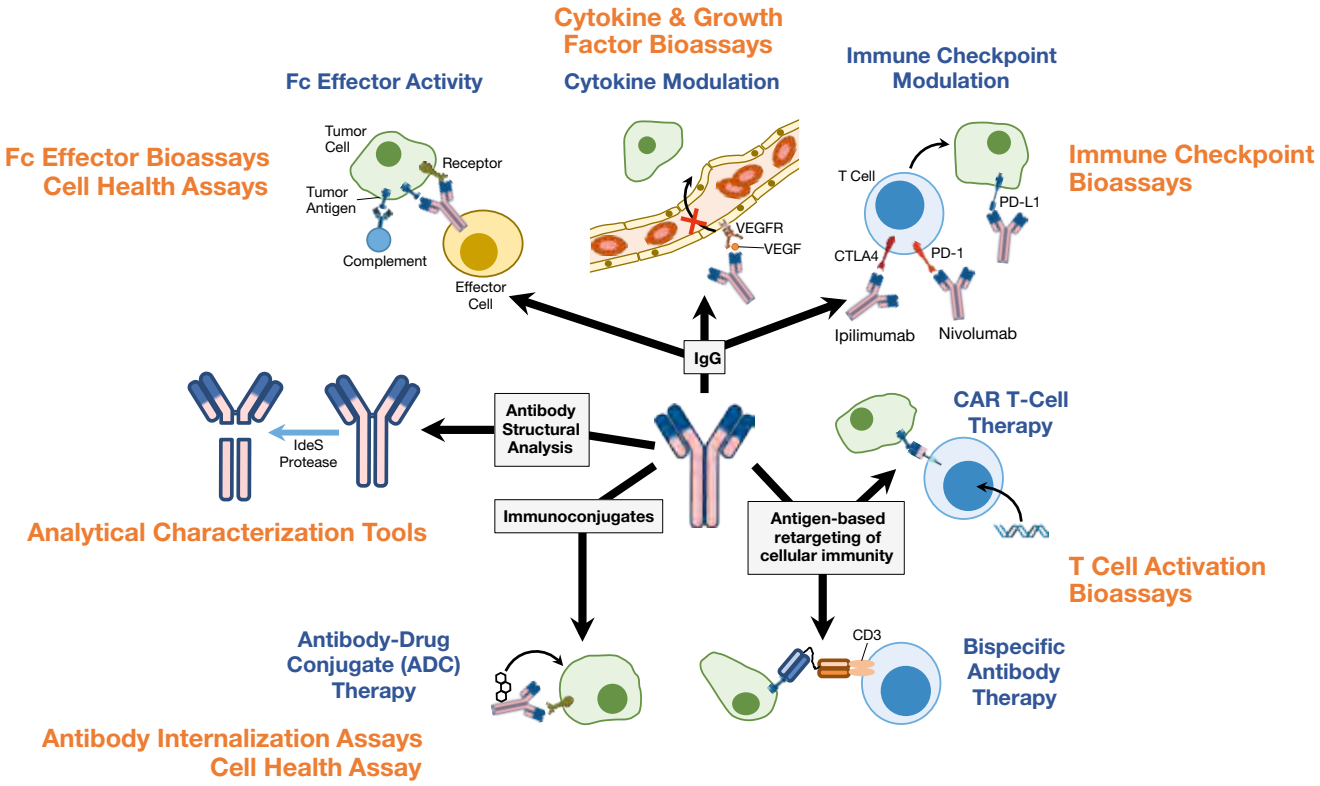
Promega

Bioassays for Biologics



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

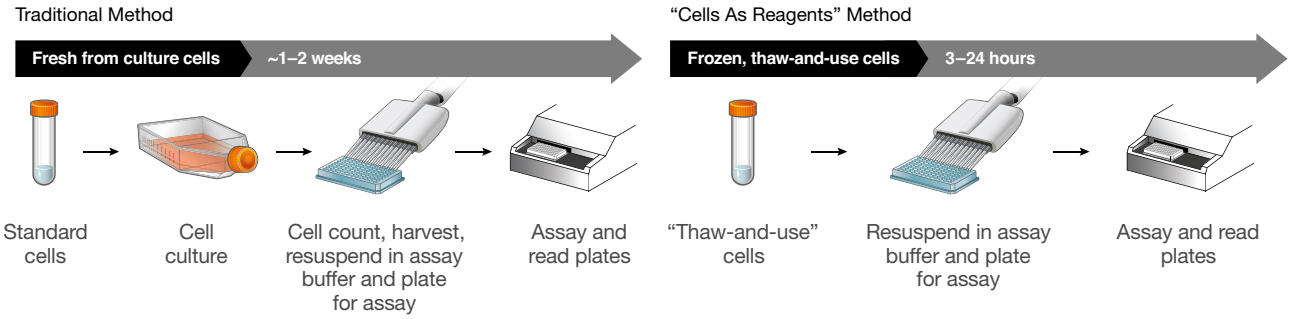
Overview

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

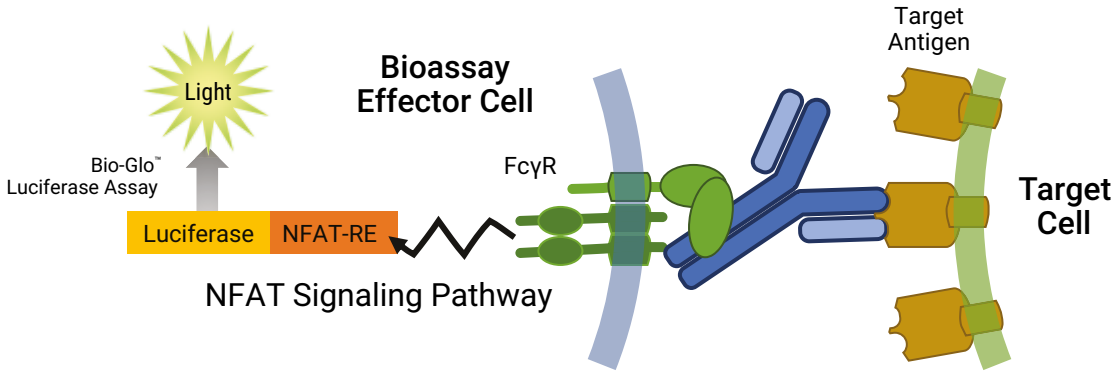
Benefits

- Cell-based reporter bioassay platform to measure ADCC and ADCP mediated through Fcγ receptors
- Cells express specific Fcγ 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 FcγRIII is the predominant receptor involved in ADCC and exists as a high-affinity (FcγRIIIa-V158) or low-affinity (FcγRIIIa-F158) variant, depending on the amino acid at position 158. Although other Fcγ receptors contribute, FcγRIIa is believed to be the predominant Fcγ 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 Fcγ receptors. The assays consist of Jurkat cells stably expressing the relevant Fcγ 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 FcγRIIa (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.

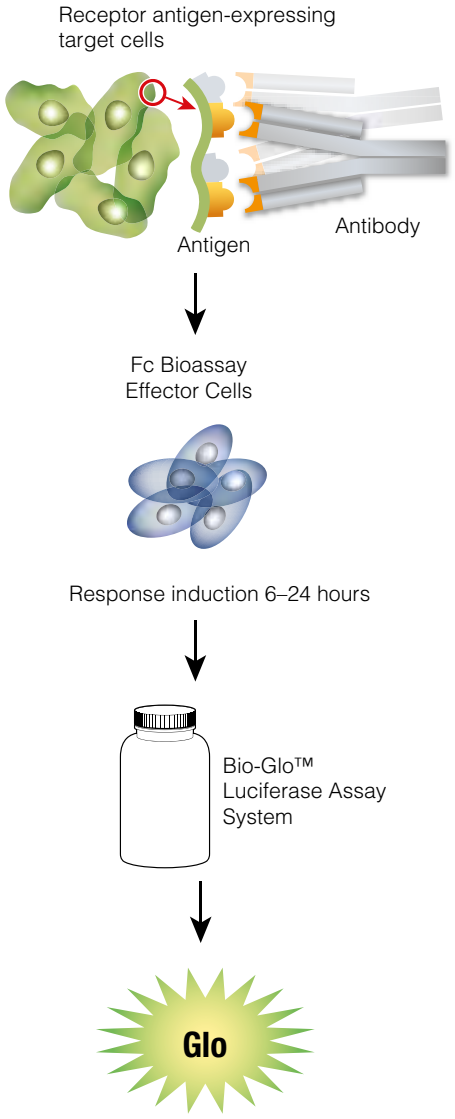
Recent Citations

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

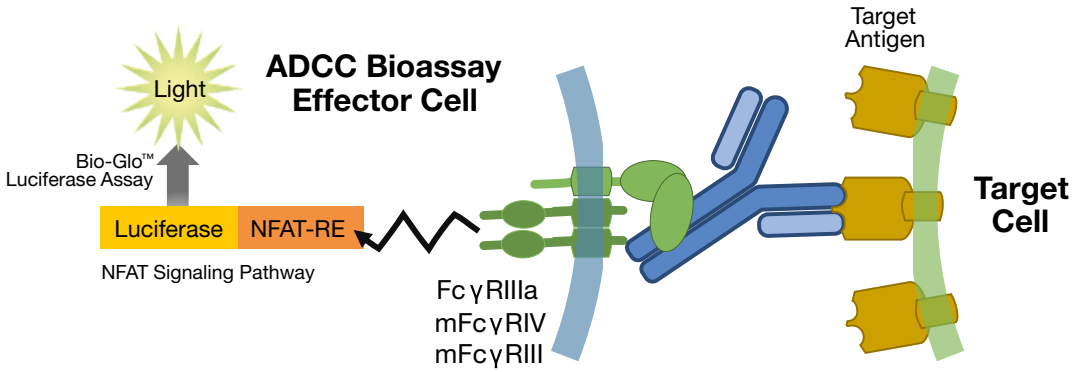
Brief pre-incubation of antigen-presenting target cells with a therapeutic antibody is followed by addition of the genetically engineered Jurkat Effector Cells. Subsequent activation of the Fc receptor signaling pathway results in increased reporter gene (luciferase) expression, measured by simple addition 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 FcγRIIIa receptor on immune system “killer” cells. Polymorphism in the FcγRIIIa receptor at amino acid 158 results in both high affinity (V158) and low affinity (F158) variants FcγRIIIa 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) FcγRIIIa variants allow quantitative measurement of the potency of therapeutic antibodies in ADCC and evaluate the impact of FcγRIIIa polymorphism in drug discovery and development.

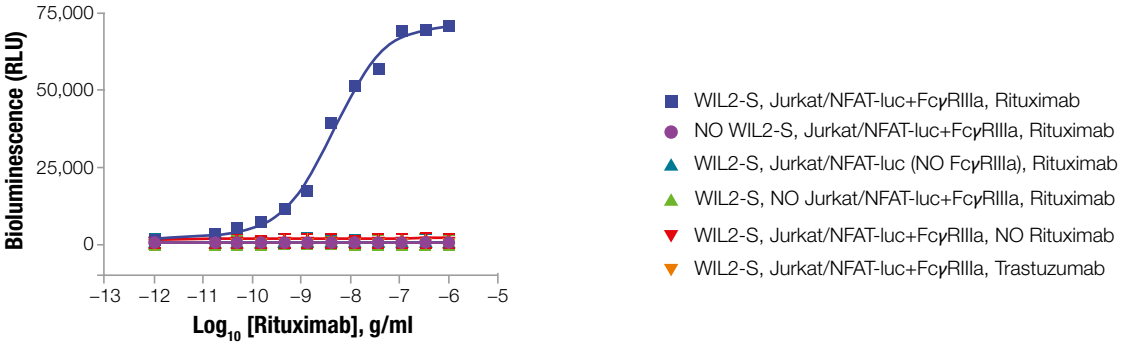
FcγRIIIa	
158 F/V or F/F	158 V/V
>85% population	~10–15% population
Less efficient antibody binding and ADCC	More efficient antibody binding and ADCC

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

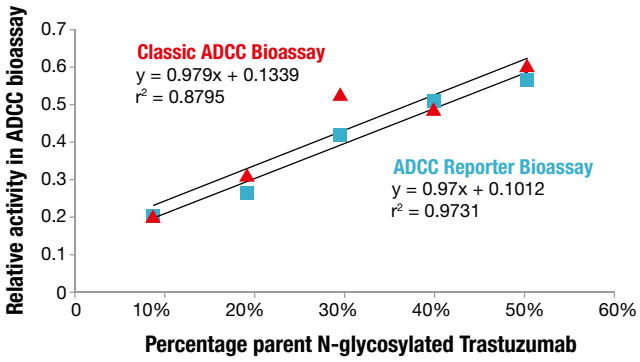
The ADCC Reporter Bioassay exhibits the distinct specificity desired for a bioassay. The reporter gene luminescent response is only present when target cells with the correct surface antigen, the correct specific antibody and effector cells expressing FcγRIIIa are present. If any of these is missing, 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

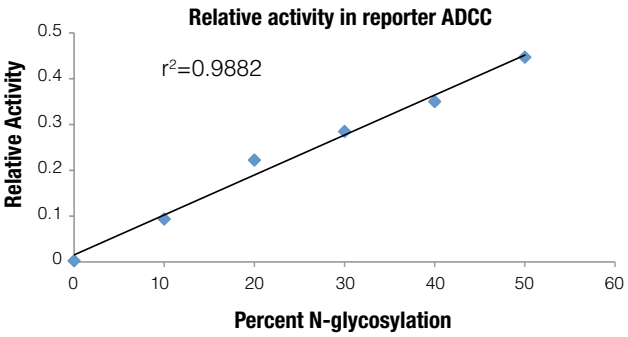
The ADCC Reporter Bioassay provides antibody activity ranking equivalent to classic LDH release ADCC Bioassay.



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

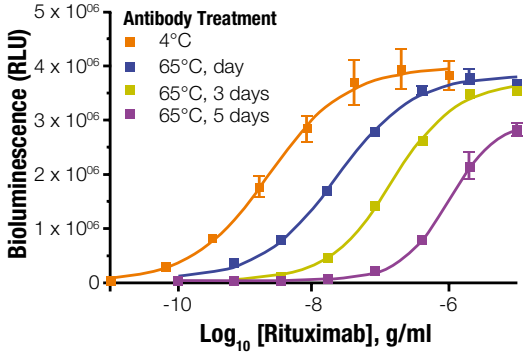
Determination of the effect of antibody glycosylation on Fc effector activity requires a robust assay to reliably detect changes in antibody potency associated with a slight change in glycosylation level. Data shown in the graph highlight that activity changes associated with small (5–10 %) changes in 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_{50}	2.13×10^{-9}	2.15×10^{-8}	1.41×10^{-7}	9.53×10^{-7}

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 1 x 1 vial ADCC Bioassay Target Cells (Raji) 1 x 5 µg Control Ab, Anti-CD20 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 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) 1 x 5 µg Control Ab, Anti-CD20	600
ADCC Reporter Bioassay, Core Kit	Catalog	G7010	1 x 1 vial ADCC Bioassay Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
ADCC Reporter Bioassay, Core Kit 5X	Catalog	G7018	5 x 1 vial ADCC Bioassay Effector Cells 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
ADCC Bioassay Effector Cells, Propagation Model	Catalog	G7102	2 x 1 vial ADCC Bioassay Effector Cells (2×10^7/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 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 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 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 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 (2×10^7/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) 1 x 1 vial FcγRIIIa-F158 Effector Cells (catalog) 1 x 1 vial FcγRIIa-H131 Effector Cells (catalog) 1 x 1 vial FcγRIIa-R131 Effector Cells (CAS) 1 x 1 vial FcγRI Effector Cells (CAS) 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	5 x 120

Mouse FcγRIV ADCC	Product Category	Cat. No.	Components	Assays in 96-well format
mFcγRIV ADCC Reporter Bioassay, Complete Kit	Catalog	M1201	1 x 1 vial mFcγRIV Effector Cells 1 x 1 vial Target Cells (Raji) 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System 1 x 1 vial Control Ab, Anti-CD20	120
mFcγRIV ADCC Reporter Bioassay, Core Kit	Catalog	M1211	1 x 1 vial mFcγRIV Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
mFcγRIV ADCC Reporter Bioassay, Core Kit 5X	Catalog	M1215	5 x 1 vial mFcγRIV Effector Cells 5X 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
mFcγRIV ADCC Bioassay Effector Cells, FcγRIV, Propagation Model	Catalog	M1212	2 x 1 vial mFcγRIV Effector Cells (2×10^7/ml)	

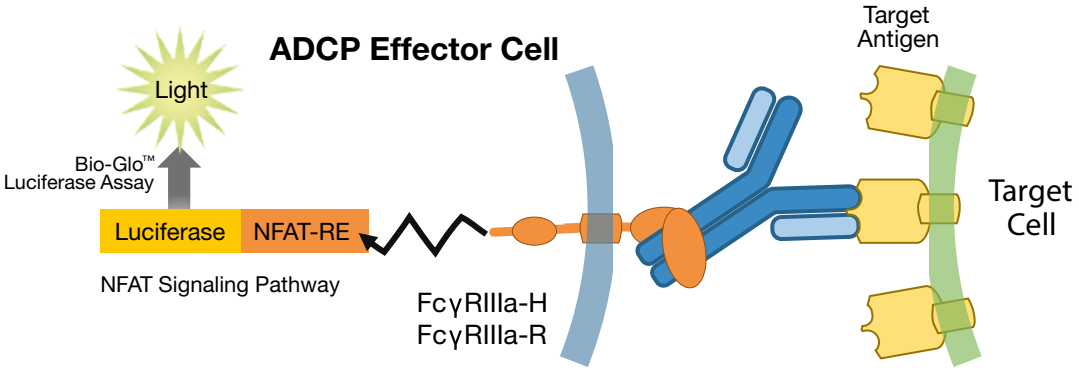
Mouse FcγRIII ADCC				
mFcγRIII ADCC Reporter Bioassay, Core Kit	CAS	CS1779B08	1 x 1 vial mFcγRIII Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 mL RMP 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
mFcγRIII ADCC Bioassay Effector Cells, Propagation Model	CAS	CS1779B06	2 x 1 vial mFcγRIII Effector Cells (2×10^7/ml)	

Bio-Glo™ Reagents				
Bio-Glo™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo™ Luciferase Assay Buffer 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 1 x 1 vial Bio-Glo™ Luciferase Assay Substrate	1000

Other Target Cells for use with ADCC Bioassays Anti-TNFα ADCC Bioassays				
Membrane TNFα Bioassay Cross-listed in the Growth Factor & Cytokine chapter	CAS	CS185502	1 x 1 vial Membrane TNFα CHO-K1 Target Cells	120
Membrane TNFα Bioassay, Propagation Model Cross-listed in the Growth Factor & Cytokine chapter	CAS	CS185501	2 x 1 vial Membrane TNFα 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



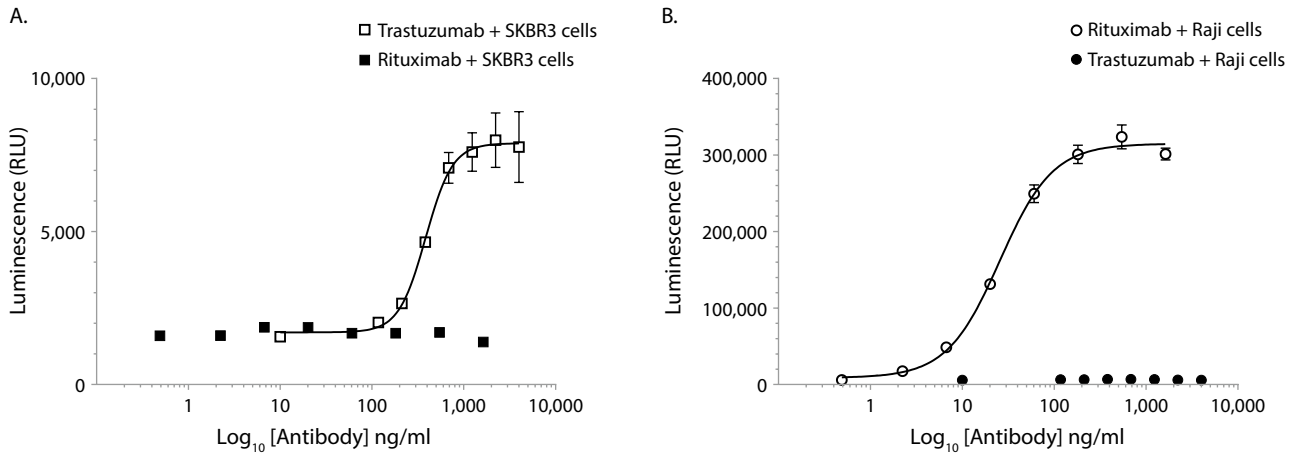
ADCP assay principle: The human ADCP Reporter Bioassays are biologically relevant MOA-based assays that can be used to measure the potency and stability of antibodies and other biologics that bind and activate the specific Fcγ receptor. The assays consist of Jurkat cells stably expressing human Fcγ and 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 FcγRIIIa expressed on NK cells, ADCP can be mediated by monocytes, macrophages, neutrophils and dendritic cells via FcγRIIa (CD32a), FcγRI (CD64) and FcγRIIIa (CD16a). The FcγRIIa-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 FcγRIIa. The assay consists of a genetically engineered Jurkat T cell line that expresses the high affinity FcγRIIa-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 FcγRIIa-R variant, that contains an arginine (R) at amino acid 131 FcγRIIa-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

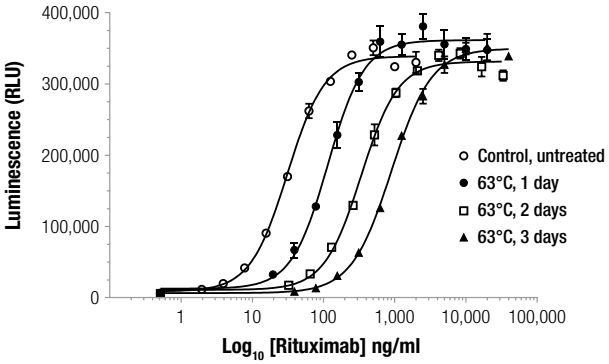
- Core Kits: include ADCC Bioassay Effector Cells, Medium, Low-IgG Serum, Bio-Glo™ Luciferase Reagent Recommended for routine use with customer-defined antibody and target cells.
- Complete Kits: include all Core Kit components, Target Cells, control antibody Recommended for use as a starter kit.

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_{50} 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. OncoImmunology 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 1 x 1 vial Target Cells (Raji) 1 x 2.5 µg ADCP Control Ab, Anti-CD20 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 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 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 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 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 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)	

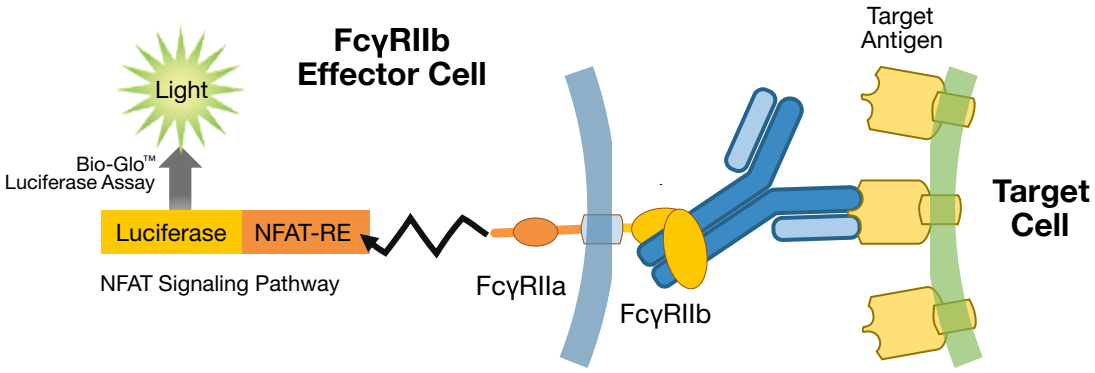
Human FcγRIIa-R Variant ADCP				
FcγRIIa-R ADCP Reporter Bioassay, Core Kit	CAS	CS1781B08	1 x 1 vial FcγRIIa-R Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
FcγRIIa-R ADCP Bioassay Effector Cells, Propagation Model	CAS	CS1781B06	2 x 1 vial FcγRIIa-R Bioassay Effector Cells (2×10^7/ml)	

Human FcγRI Variant ADCP				
FcγRI ADCP Reporter Bioassay, Core Kit	CAS	CS1781C08	1 x 1 vial FcγRI Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
FcγRI ADCP Reporter Bioassay, Core Kit 5X	CAS	CS1781C10	5 x 1 vial FcγRI Effector Cells 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo Luciferase Assay System	600
FcγRI ADCP Bioassay Effector Cells, Propagation Model	CAS	CS1781C06	2 x 1 vial FcγRI ADCP Bioassay (2×10^7/ml)	

Bio-Glo™ Reagents				
Bio-Glo™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo™ Luciferase Assay Buffer 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 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.

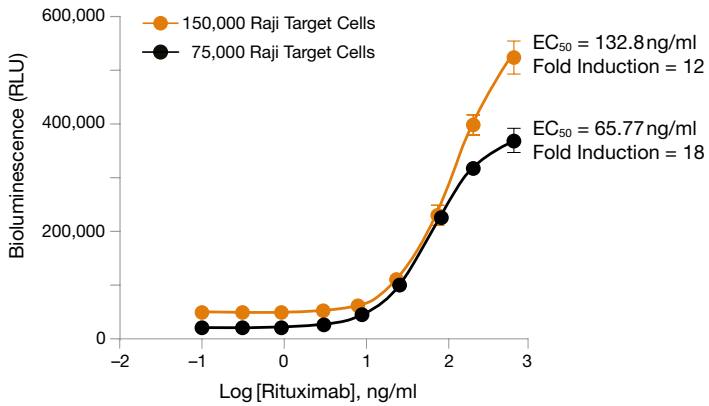
Human FcγRIIb Bioassay



FcγRIIb assay principle: Activation of FcγRIIb transmits a signal through the FcγRIIa 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 FcγRIIb/RIIa chimera will transduce intracellular signals resulting in NFAT-mediated luciferase activity that can be easily quantified.

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

Activation of FcγRIIb/FcγRIIa Chimera by Rituximab



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

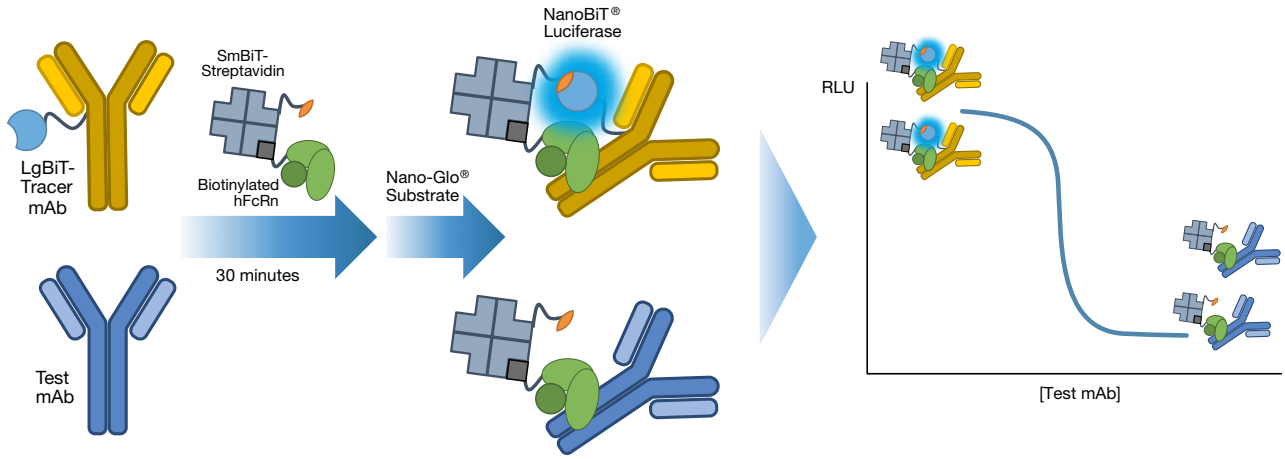
Human FcγRIIb Bioassay: Ordering information

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 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
FcγRIIb Bioassay, Core Kit 5X	CAS	CS1781E04	5 x 1 vial FcγRIIb Effector Cells 5 x 4 ml Low IgG Serum 5 x 36 ml RPMI 1640 Medium 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 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 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

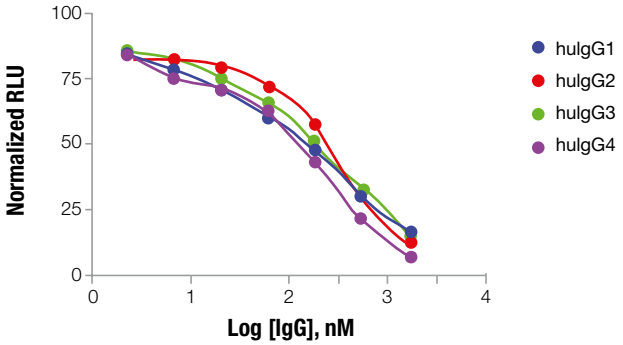


NanoBiT FcRn competition assay principle

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.

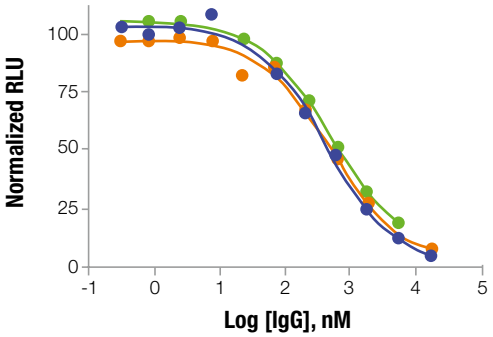
Lumit™ FcRn Binding Immunoassay is a homogeneous (no-wash) competition assay to measure the interaction between human FcRn and Fc proteins including antibodies. Human IgG labeled with LgBiT (hIgG-LgBiT) is used as a tracer. A C-terminus biotinylated FcRn attached to Streptavidin-SmBiT (FcRn-SAv-SmBiT) 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



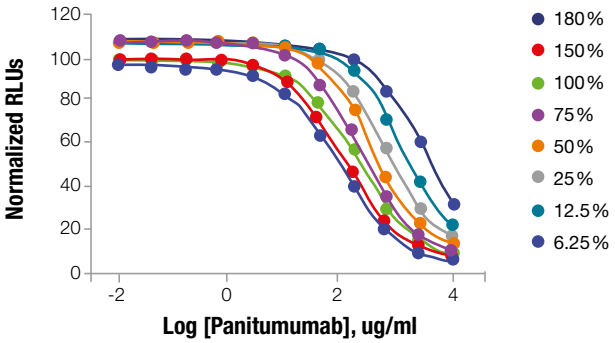
The Lumit™ FcRn Binding Immunoassay can detect FcRn binding affinity for the four major types of human antibody isotypes.

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 repsonse 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 1 x Streptavidin-SmBiT 1 x Tracer-LgBiT Antibody (hIgG1) 1 x FcRn Assay Buffer 1 x pH Adjustment Buffer 1 x Lumit™ Detection Substrate A 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

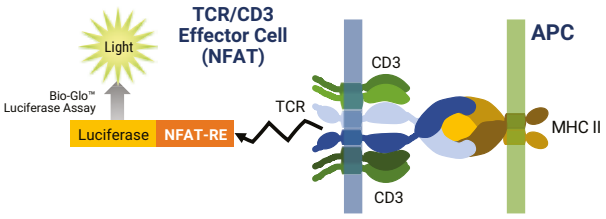
Overview

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-κB pathways, thereby inducing IL-2 production.

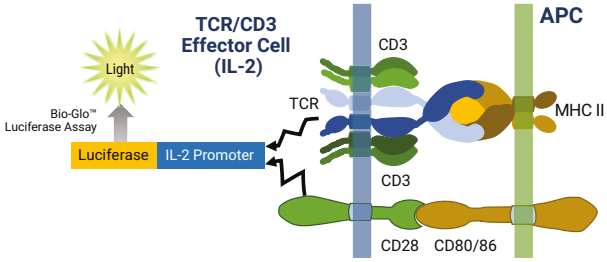
The T Cell Activation Bioassays are bioluminescent cell-based assays that overcome limitations of inconsistencies posed by existing assays. They can be used for the discovery and development of anti-CD3 bispecific molecules and to evaluate the activity of exogenously expressed chimeric antigen receptor (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)

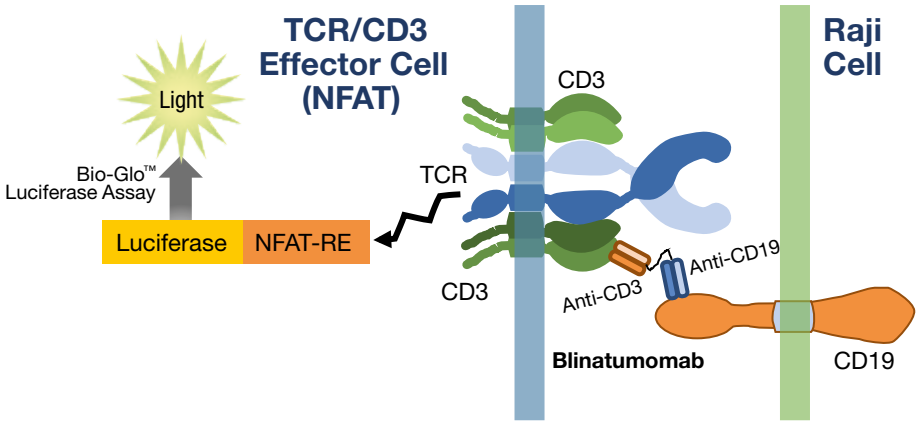


T Cell Activation Bioassay (IL-2)



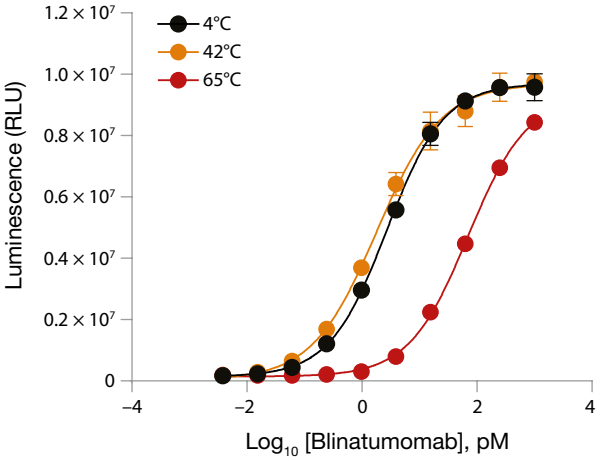
T Cell Activation Bioassay principle. The assay consists of a genetically engineered Jurkat T cell line that expresses a luciferase reporter (TCR/CD3 Effector Cells) driven by either an NFAT-response element (NFAT-RE) or an IL-2 promoter. When the TCR/CD3 Effector Cells (NFAT) are engaged with an appropriate 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

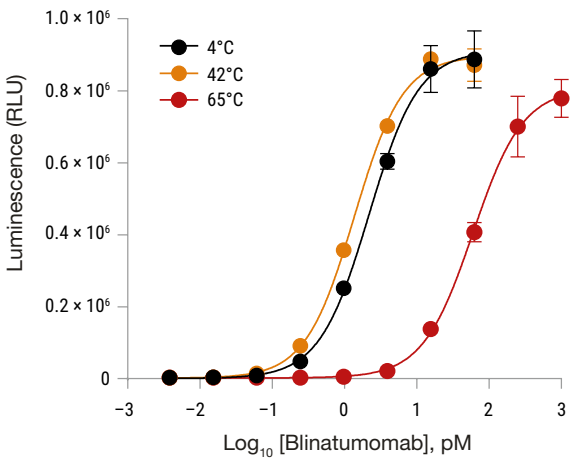


Measuring Blinatumomab (CD3 × CD19 BiTE) activity. When the TCR/CD3 Effector Cells (NFAT) are engaged with an appropriate TCR/CD3 ligand or anti-TCR/CD3 antibody, the TCR transduces intracellular signals resulting in NFAT-RE-mediated luminescence.

T Cell Activation Bioassay (NFAT)

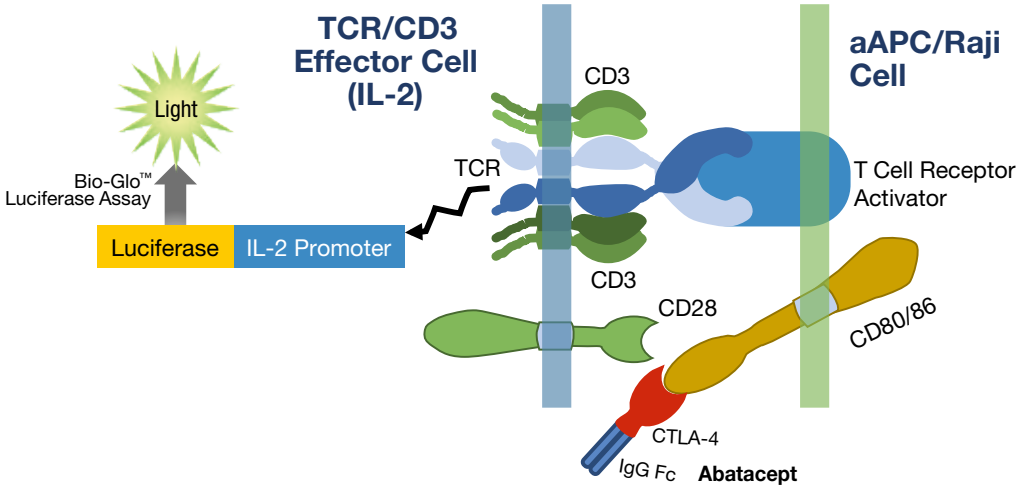


T Cell Activation Bioassay (IL-2)

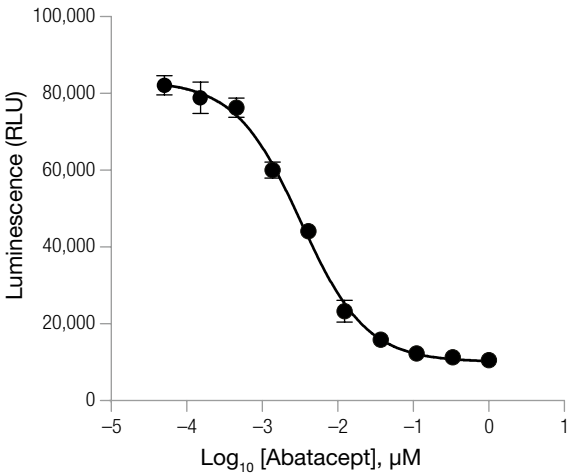


The T Cell Activation Bioassay indicates the relative stability of antibodies. Samples of Blinatumomab anti-CD3 and anti-CD19 bispecific antibody were stored at 4°C or heat-treated (42°C or 65°C). The antibodies were analyzed using the T Cell Activation Bioassay (NFAT or IL-2, as indicated).

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

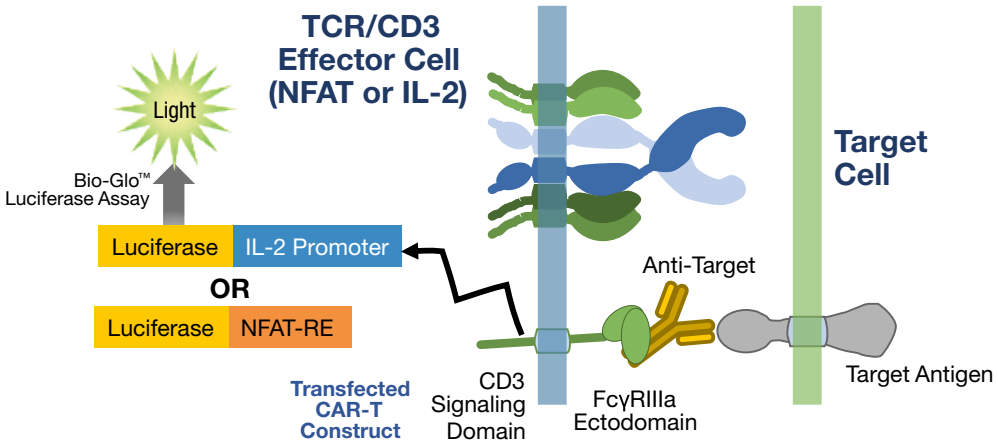


Blockade of CD28:CD80/86 with TCR engagement. Abatacept prevents co-stimulation of IL-2 promoter by CD28:CD80/86 engagement



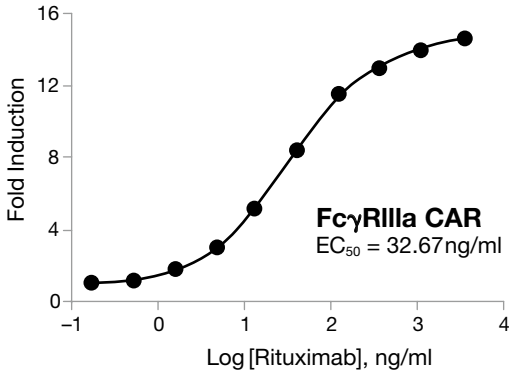
MOA-reflecting specificity. TCR/CD3 Effector Cells (IL-2) were incubated with increasing concentrations of Abatacept in the presence of an artificial antigen presenting cell (aAPC) consisting of Raji (CD80/CD86+) cells expressing an engineered cell surface protein designed to activate cognate TCRs in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

Measuring the Activity of CAR-T Cell Receptors

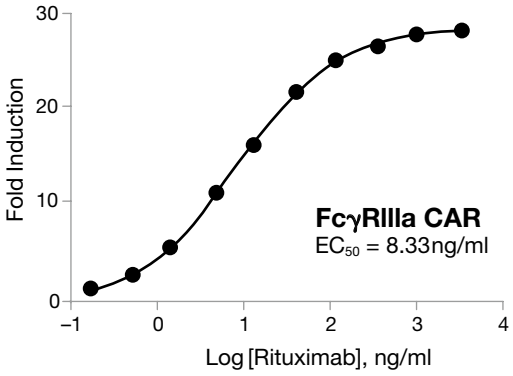


Measurement of Chimeric Antigen Receptor T (CAR-T) Cell Activity. TCR/CD3 (NFAT or IL-2) effector cells were transiently transfected with a chimeric antigen receptor.

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) 1 x 36 ml RPMI 1640 Medium 1 x 4 ml Fetal Bovine Serum 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) 5 x 36 ml RPMI 1640 Medium 5 x 4 ml Fetal Bovine Serum 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) 1 x 36 ml RPMI 1640 Medium 1 x 4 ml Fetal Bovine Serum 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) 5 x 36 ml RPMI 1640 Medium 5 x 4 ml Fetal Bovine Serum 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 *(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 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 1 x 1 vial Bio-Glo™ Luciferase Assay Substrate	1000
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer 1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate	100
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7940	1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer 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

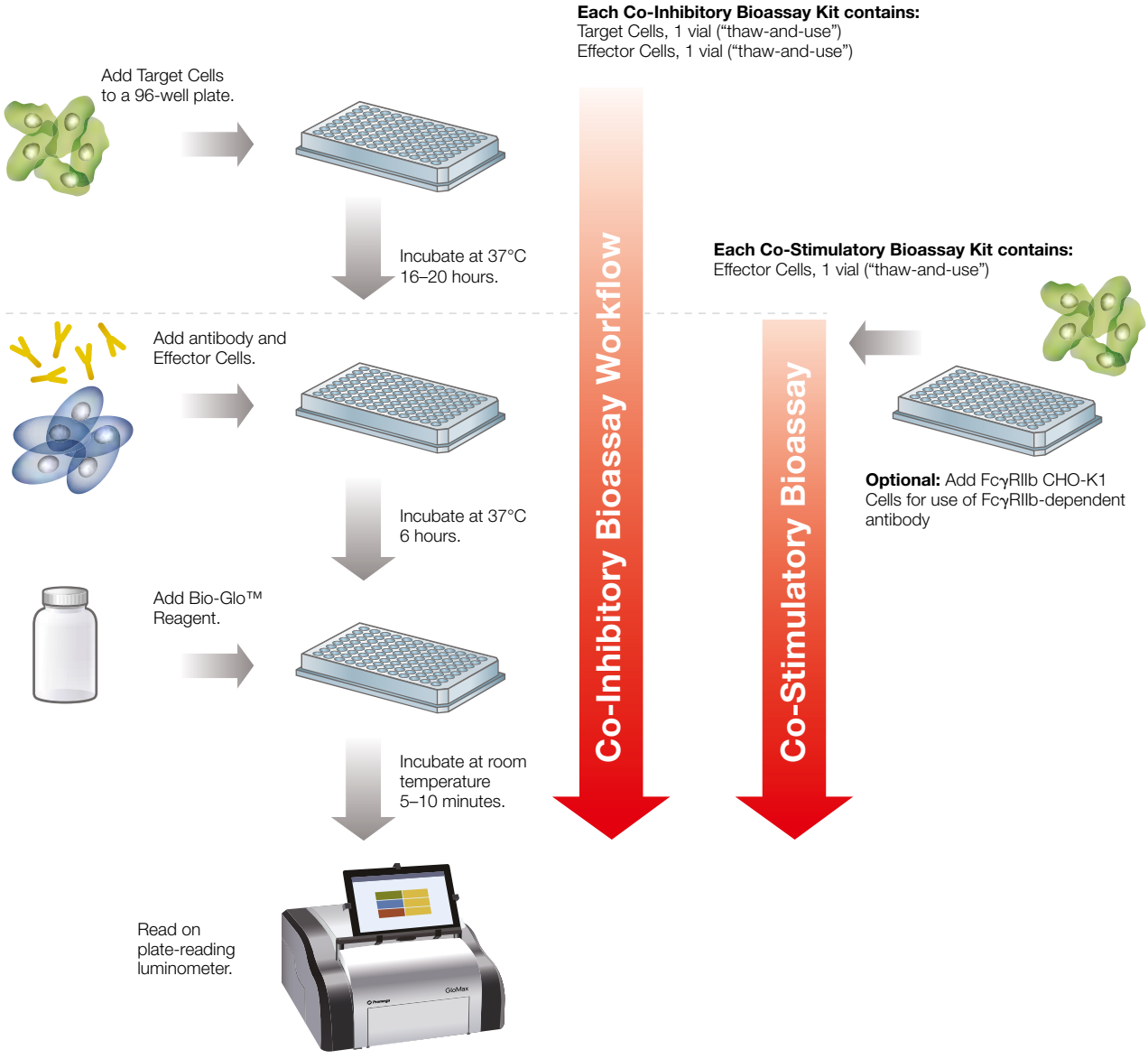
Immune inhibitory receptors (e.g. PD-1, CTLA-4, TIGIT, LAG-3) expressed on activated T cells and B cells play a critical role in regulating immune responses to tumor antigens and autoantigens. Engagement of a receptor by its ligand on an adjacent cell inhibits T cell receptor (TCR) signaling and TCR-mediated 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 FcγR-mediated crosslinking, which is therapeutically relevant *in vivo.*

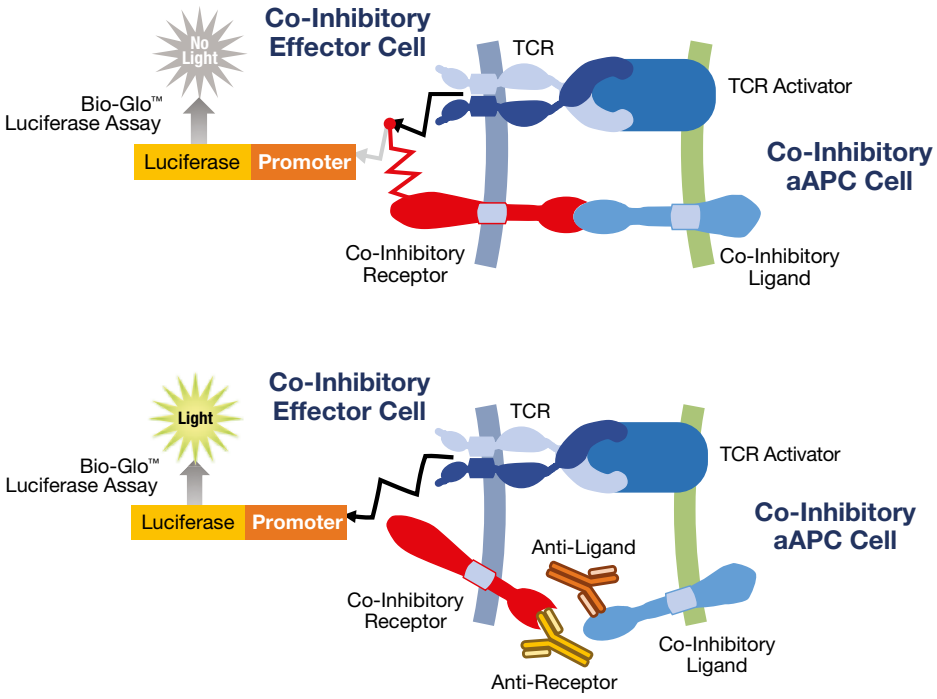
All Bioassays are available as “thaw-and-use” kits in 1X and 5X sizes including all required reagents in standardized formats or as Cell propagation model.

Schematic protocol for the Immune Checkpoint Bioassays

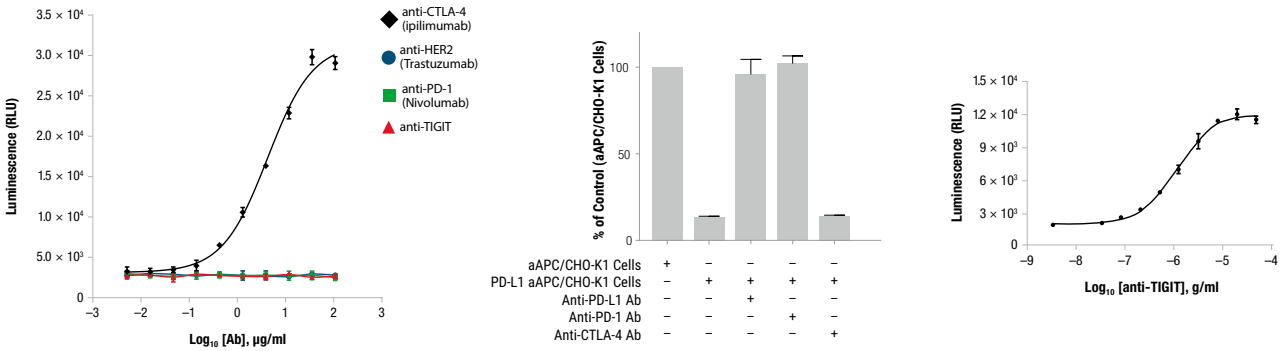


The Immune Checkpoint Bioassays combine a simple, add-mix-read single-day workflow provided in a frozen, "thaw-and-use" format and an optimized protocol that exhibits low variability and high accuracy. No cell culture is required. For some co-stimulatory antibodies it might be beneficial to test the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

Co-Inhibitory Receptor Bioassays

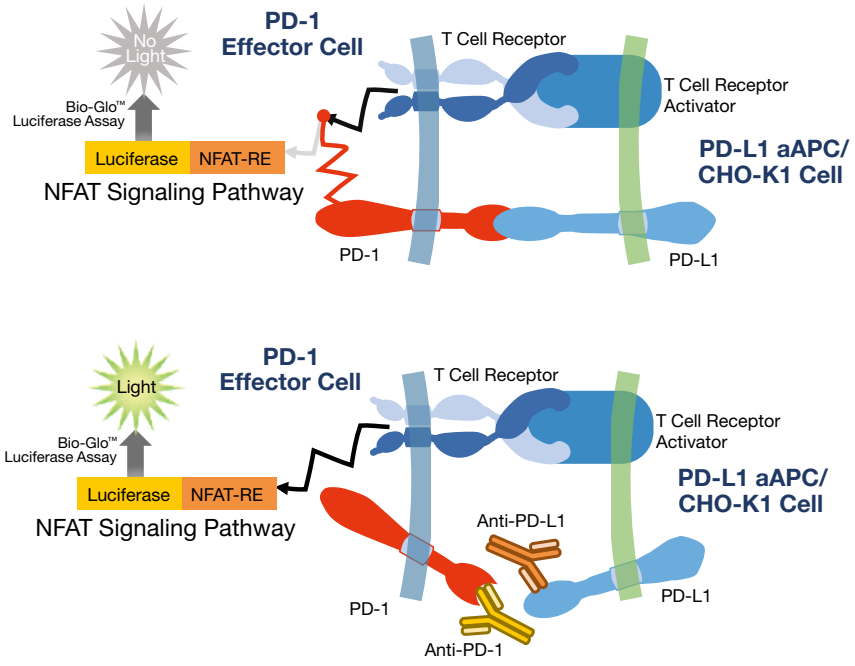


Co-inhibitory bioassay principle. The bioassay typically consists of two genetically engineered cell lines: 1) an effector cell that expresses the receptor; and 2) an artificial antigen-presenting cell (aAPC) that expresses the ligand. During co-culture, the TCR is activated by the TCR activator (an 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_{50} and fold induction indicating potential for further development into a neutralizing antibody bioassay.

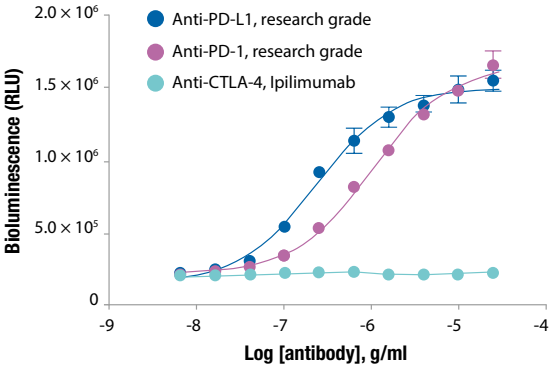
The assay consists of two genetically engineered cell types:

- **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:

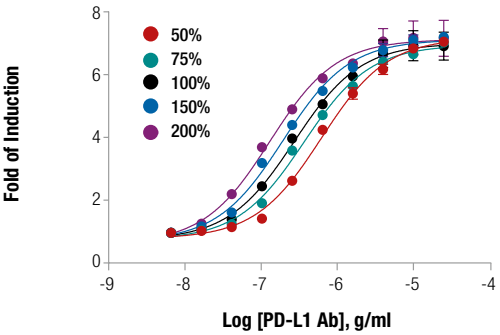
1. Finlay, W.J.J., et al. (2019) Anti-PD1 'SHR-1210' aberrantly targets pro-angiogenic receptors and this polyspecificity can be ablated by paratope refinement. mAbs11, 26–44.
2. Boohaker, R.J., et al. (2018) Rational design and development of a peptide inhibitor for the PD-1/PD-L1 interaction. Cancer Lett. 434, 11–21.
3. Clarke, A.W., et al. (2018) An anti-TL1A antibody for the treatment of asthma and inflammatory bowel disease. mAbs10, 664–77.

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



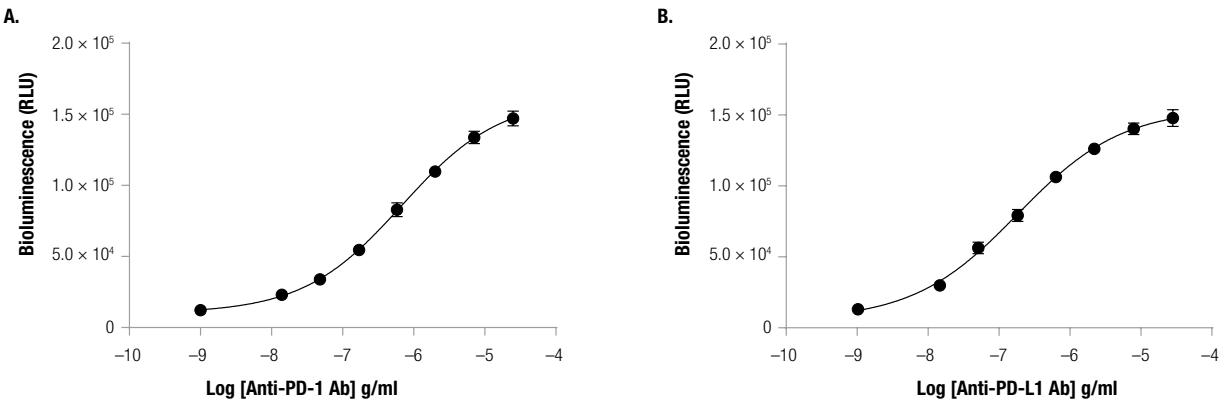
PD-1 Effector Cells and PD-L1 aAPC Cells were incubated for 6 hrs at 37°C with increasing concentrations of either an anti-PD-1, PD-L1 or CTLA-4 antibody. The anti-PD-1 and PD-L1 antibodies, but not the anti-CTLA-4 antibody, blocked the immune checkpoint inhibitory signal resulting in luciferase activity.

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



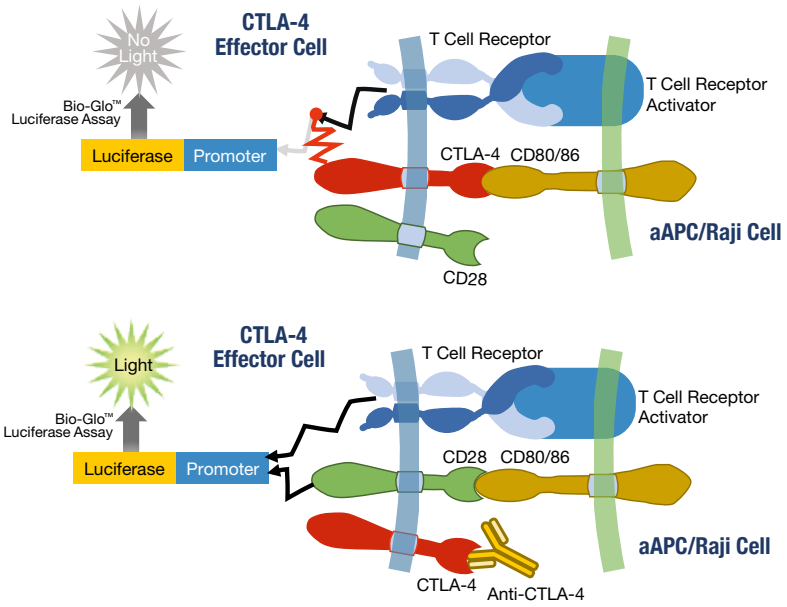
PD-1 Effector Cells and PD-L1 aAPC Cells were incubated with increasing concentrations of anti-PD-L1 antibody preparations representing a 50–200 % potency range. The PD-1/PD-L1 Blockade Bioassay demonstrated appropriate rank ordering of antibody potency.

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.

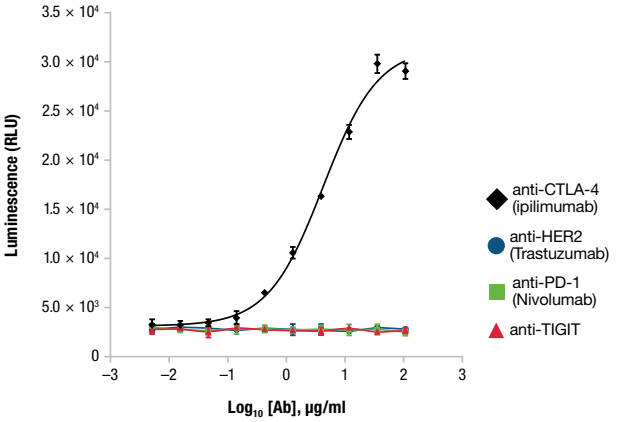
The assay consists of two genetically engineered cell types:

- **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:

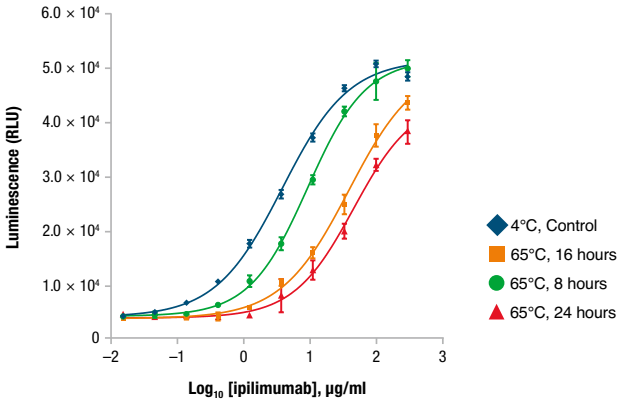
1. Duperret, E.K., et al. (2018) Synthetic DNA-encoded monoclonal antibody delivery of anti-CTLA-4 antibodies induces tumor shrinkage *in vivo.* Cancer Res. 78, 6363–70.
2. Gombos, R.B., et al. (2018) Toxicological and pharmacological assessment of AGEN1884, a novel human IgG1 anti-CTLA-4 antibody. PLoS 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.



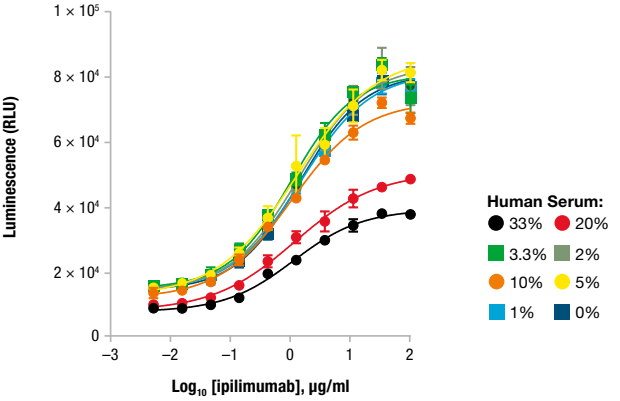
CTLA-4 Effector Cells were incubated with aAPC/Raji Cells in the absence or presence of anti-CTLA-4, anti-PD-1, anti-TIGIT or anti-HER2 blocking antibodies, as indicated.

Stability Indication



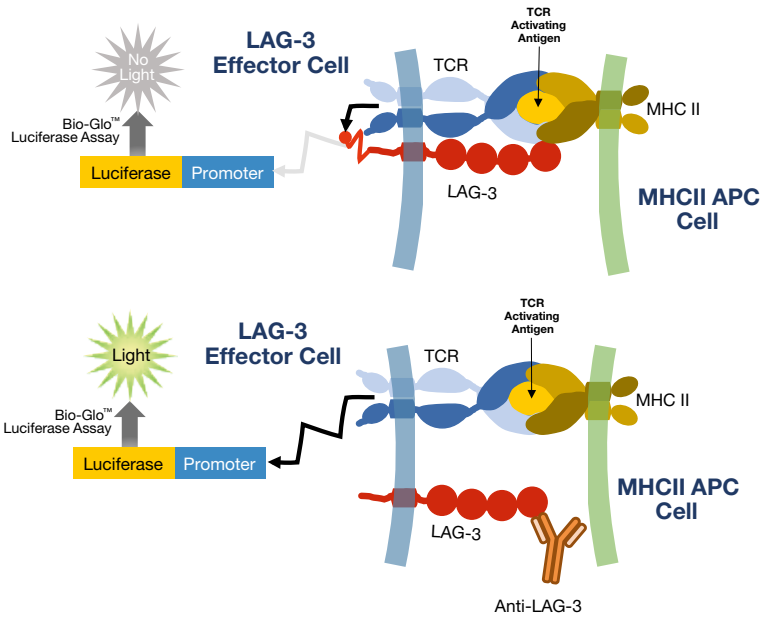
Samples of anti-CTLA-4 antibody, ipilimumab, were maintained at 4°C (control) or heat-denatured at 65°C for the indicated times and analyzed using the CTLA-4 Blockade Bioassay.

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+ and CD8+ 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.

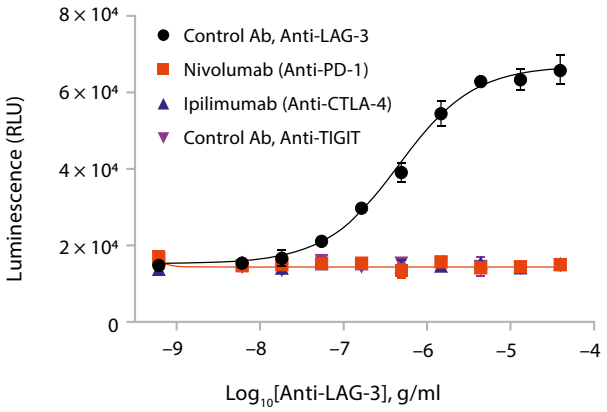
The assay consists of two genetically engineered cell types:

- **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:

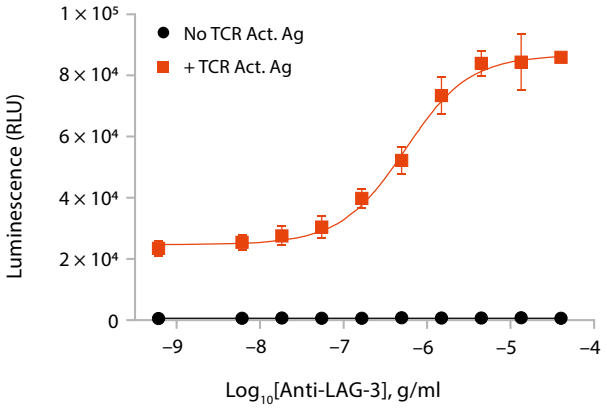
1. Ghosh, S., et al. (2019) TSR 033, a novel therapeutic antibody targeting LAG-3, enhances T cell function and the activity of PD-1 blockade *in vitro* and *in vivo.* Mol. Cancer Ther.

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



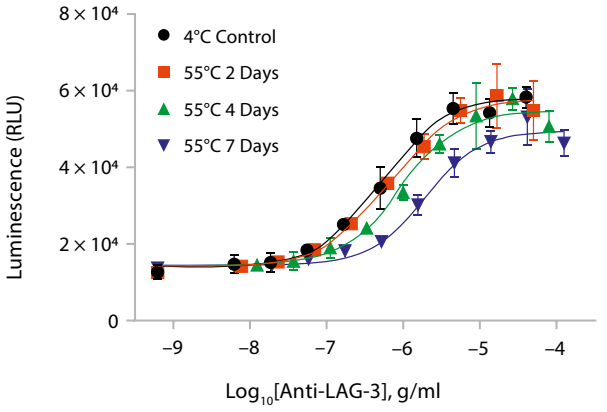
LAG-3 Effector Cells were incubated with TCR Activating Antigen and MHCII APC cells in the presence of anti-LAG-3, anti-PD-1, anti-CTLA-4 or anti-TIGIT antibodies, as indicated.

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



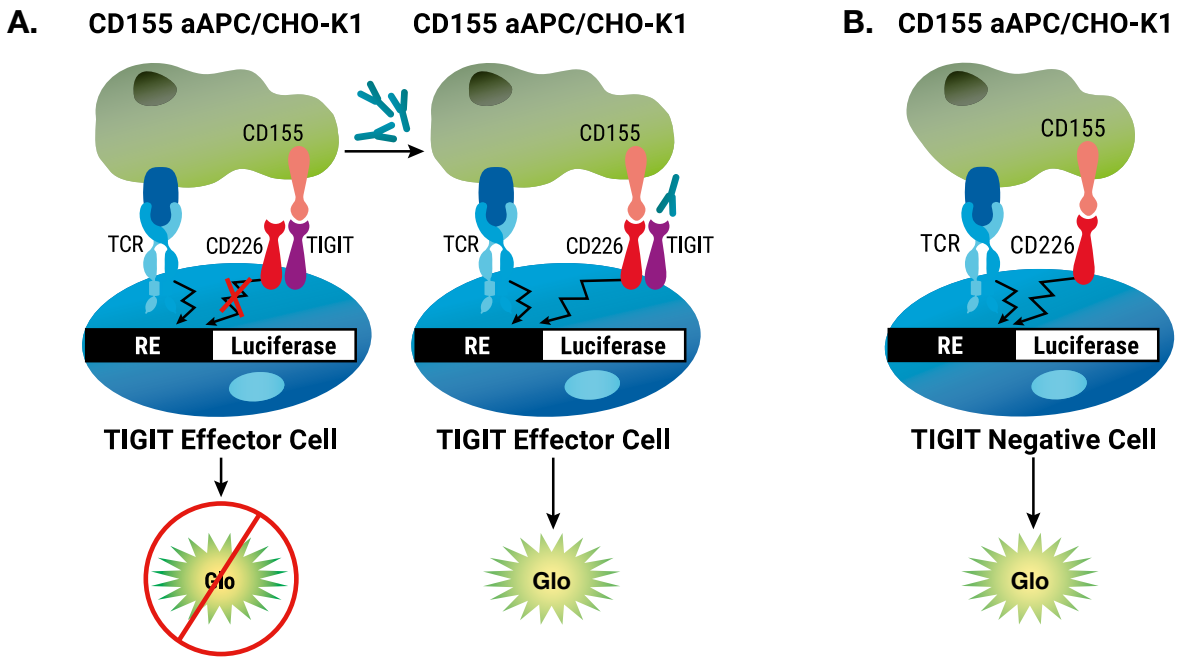
MHCII APC Cells were plated with and without TCR Activating Antigen. The next day, a titration of Anti-LAG-3 was plated followed by addition of LAG-3 Effector Cells. After a 5-hour induction, Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Discover Detection System.

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



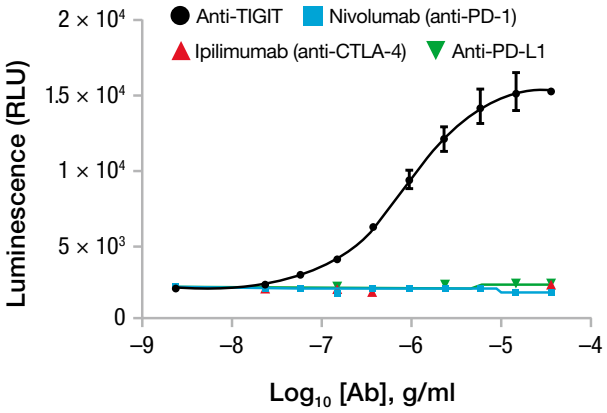
Representation of the TIGIT/CD155 Blockade Bioassay. The bioassay consists of two genetically engineered cell lines, TIGIT Effector Cells and CD155 aAPC/CHO-K1 Cells. **A.** When co-cultured, TIGIT inhibits CD226 pathway-activated luminescence. The addition of anti-TIGIT antibody blocks the TIGIT/CD155 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+ and CD8+ 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:

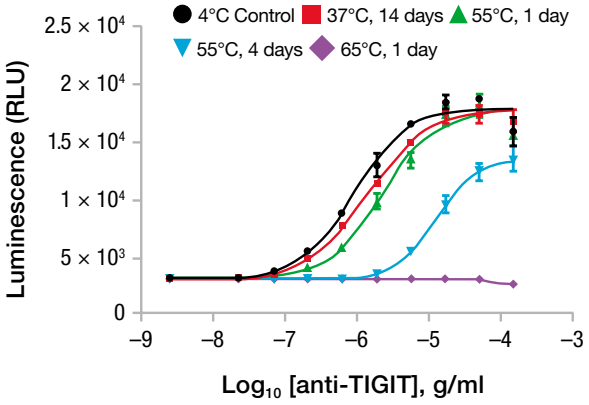
- **TIGIT Effector Cells:** Jurkat T cells expressing human TIGIT with a luciferase reporter driven by a native promoter that can respond to both TCR activation and CD226 co-stimulation.
- **CD155 aAPC/CHO-K1 Cells:** CHO-K1 cells engineered to express human CD155 with an engineered cell-surface protein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



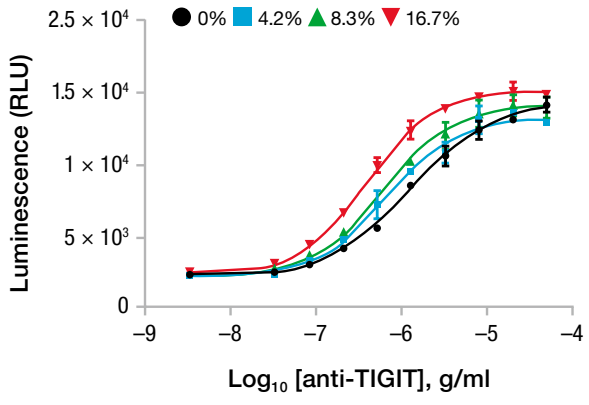
TIGIT Effector Cells were incubated with CD155 aAPC/CHO-K1 Cells in the presence of a serial titration of Anti-TIGIT, anti-PD-1 (Nivolumab), anti-CTLA-4 (Ipilimumab) or anti-PD-L1-blocking antibodies as indicated. Bio-Glo™ Reagent was added and luminescence quantified.

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
PD-1/PD-L1 Blockade Bioassay				
PD-1/PD-L1 Blockade Bioassay	Catalog	J1250	1 x 1 vial PD-1 Effector Cells 1 x 1 vial PD-L1 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x Ham's F12 Medium 1 x 36 ml RPMI 1640 Medium 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 5 x 1 vial PD-L1 aAPC/CHO-K1 Cells 5 x 4 ml Fetal Bovine Serum 5 x Ham's F12 Medium 5 x 36 ml RPMI 1640 Medium 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 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	
PD-1/PD-L2 Blockade Bioassay				
PD-1/PD-L2 Blockade Bioassay Kit	CAS	CS187131-1	1 x 1 vial PD-1 Effector Cells 1 x 1 vial PD-L2 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x Bio-Glo™ Luciferase System 1 x 36 ml RPMI Medium 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 5 x 1 vial PD-L2 aAPC/CHO-K1 Cells 5 x 4 ml Fetal Bovine Serum 5 x Bio-Glo™ Luciferase System 5 x 36 ml RPMI Medium 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 2 x 1 vial PD-L2 Effector Cells aAPC/CHO-K1 Cells	
Mouse PD-1/PD-L1 Blockade Bioassay				
Mouse PD-1/PD-L1 Blockade Bioassay	CAS	CS303201	1 x 1 vial Mouse PD-1 Effector Cells 1 x 1 vial Mouse PD-L1 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x Ham's F12 Medium 1 x 36 ml RPMI 1640 Medium 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 5 x 1 vial Mouse PD-L1 aAPC/CHO-K1 Cells 5 x 4 ml Fetal Bovine Serum 5 x Ham's F12 Medium 5 x 36 ml RPMI 1640 Medium 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 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
CTLA-4 Blockade Bioassay				
CTLA-4 Blockade Bioassay Kit	CAS	JA3001	CTLA-4 Effector Cells and aAPC/Raji Cells 1 x 1 vial CTLA-4 Effector Cells 1 x 1 vial aAPC/Raji Cells 4 ml Fetal Bovine Serum 1 x 36ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
CTLA-4 Blockade Bioassay Kit 5X	CAS	JA3005	CTLA-4 Effector Cells and aAPC/Raji Cells 5 x 1 vial CTLA-4 Effector Cells 5 x 1 vial aAPC/Raji Cells 5 x 4 ml Fetal Bovine Serum 5 x 36ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
CTLA-4 Blockade Bioassay, Propagation Model	CAS	JA1400	CTLA-4 Effector Cells aAPC/Raji Cells	
Control Ab (Anti-CTLA-4)	CAS	JA1020	1 x 20 µg Control Ab (Anti-CTLA-4)	
LAG-3 Blockade Bioassay				
LAG-3 Blockade Bioassay Kit	Catalog	JA1111	1 x 1 vial LAG-3 Effector Cells 1 x 1 vial MHCII APC Cells 1 x 4 ml Fetal Bovine Serum 1 x 60 ml DMEM Medium 1 x 36 ml RPMI 1640 Medium 1 x 0.06 ml TCR Activating Antigen 1 x Bio-Glo™ Luciferase Assay System	120
LAG-3 Blockade Bioassay Kit 5X	Catalog	JA1115	5 x 1 vial LAG-3 Effector Cells 5 x 1 vial MHCII APC Cells 5 x 4 ml Fetal Bovine Serum 5 x 60 ml DMEM Medium 5 x 36 ml RPMI 1640 Medium 5 x 0.06 ml TCR Activating Antigen 5 x Bio-Glo™ Luciferase Assay System	600
LAG-3 Blockade Bioassay, Propagation Model	Catalog	JA1112	2 x 1 vial LAG-3 Effector Cells 2 x 1 vial MHCII APC Cells 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)	
TIGIT/CD155 Blockade Bioassay				
TIGIT/CD155 Blockade Bioassay Kit	Catalog	J2201	1 x 1 vial TIGIT Effector Cells 1 x 1 vial CD155 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
TIGIT/CD155 Blockade Bioassay Kit 5X	Catalog	J2205	5 x 1 vial TIGIT Effector Cells 5 x 1 vial CD155 aAPC/CHO-K1 Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
TIGIT/CD155 Blockade Bioassay, Propagation Model	Catalog	J2092	2 x 1 vial TIGIT Effector Cells 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
CD226/CD155 Blockade Bioassay				
CD226/CD155 Blockade Bioassay Kit	CAS	CS1978F01	1 x 1 vial TCR/CD3 Effector Cells (IL-2) 1 x 1 vial CD155 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 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) 2 x 1 vial CD155 aAPC/CHO-K1 Cells	
TIM-3 Bioassay *(utilizes NanoLuc® Luciferase)*				
TIM-3 Bioassay Kit	Catalog	JA2211	1 x 1 vial TIM-3 Effector Cells 1 x 1 vial TIM-3 Target Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo-NL™ Luciferase Assay System	120
TIM-3 Bioassay Kit 5X	Catalog	JA2215	5 x 1 vial TIM-3 Effector Cells 5 x 1 vial TIM-3 Target Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo-NL™ Luciferase Assay System	600
TIM-3 Bioassay, Propagation Model	Catalog	JA2222	2 x 1 vial TIM-3 Effector Cells 2 x 1 vial TIM-3 Target Cells	
Control Ab (Anti-TIM-3)	Catalog	K1210	1 x 100 µg Control Ab (Anti-TIM-3)	
BTLA/HVEM Blockade Bioassay				
BTLA/HVEM Blockade Bioassay Kit	CAS	CS1978E04	1 x 1 vial BTLA Effector Cells 1 x 1 vial HVEM aAPC/CHO-K1 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
BTLA/HVEM Blockade Bioassay Kit 5X	CAS	CS1978E08	5 x 1 vial BTLA Effector Cells 5 x 1 vial HVEM aAPC/CHO-K1 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
BTLA/HVEM Blockade Bioassay, Propagation Model	CAS	CS1978E01	2 x 1 vial BTLA Effector Cells 2 x 1 vial HVEM aAPC/CHO-K1 Cells	

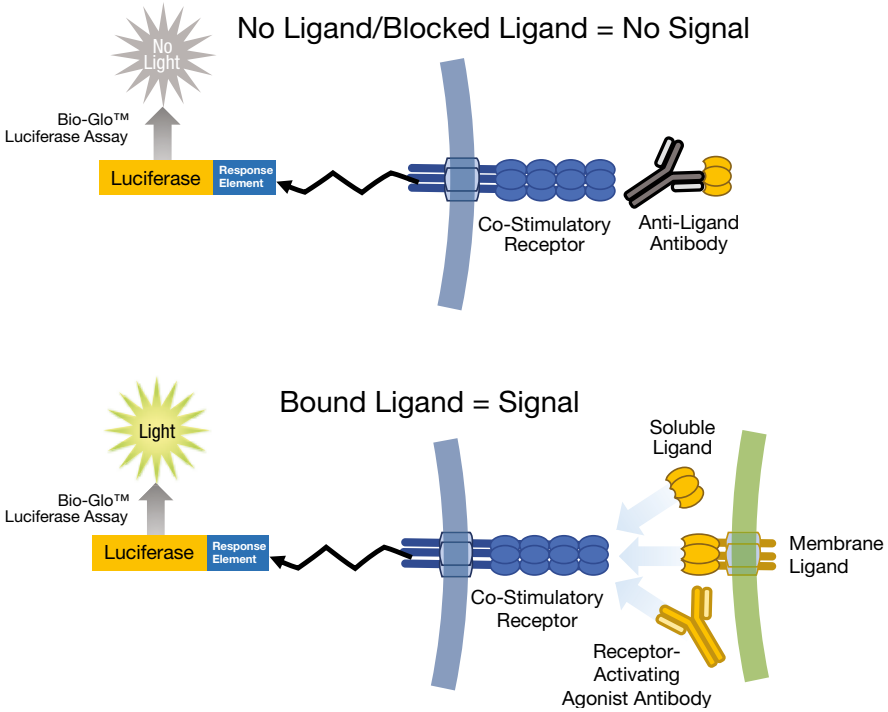
Co-Inhibitory Receptor Bioassays	Product Category	Cat. No.	Components	Assays in 96-well format
ICOS Blockade Bioassay *(utilizes NanoLuc® Luciferase)* *The ICOS Bioassay is available in both BLOCKADE and AGONIST formats. The AGONIST products are listed in the 'Co-Stimulatory Bioassays' section.*				
ICOS Blockade Bioassay Kit	CAS	CS1979D16	1 x 1 vial ICOS Effector Cells 1 x 1 vial ICOSL aAPC/CHO-K1 1 x 4 ml Fetal Bovine Serum 1 x 36ml RPMI 1640 Medium 1 x 25ml Ham's F12 Medium 1 x Bio-Glo-NL™ Luciferase Assay System	120
ICOS Blockade Bioassay Kit 5X	CAS	CS1979D20	5 x 1 vial ICOS Effector Cells 5 x 1 vial ICOSL aAPC/CHO-K1 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x 25 ml Ham's F12 Medium 5 x Bio-Glo-NL™ Luciferase Assay System	600
ICOS Blockade Bioassay, Propagation Model	CAS	CS1979D08	2 x 1 vial ICOS Effector Cells 2 x 1 vial ICOSL aAPC/CHO-K1 Cells	

CD28 Blockade Bioassay				
The CD28 Bioassay is available in both BLOCKADE and AGONIST formats. The AGONIST products are listed in the 'Co-Stimulatory Bioassays' section.				
CD28 Blockade Bioassay Kit	CAS	CS1979C10	1 x 1 vial TCR/CD3 Effector Cells (IL-2) 1 x 1 vial aAPC/Raji Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x 25 ml Ham's F12 Medium 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) 5 x 1 vial aAPC/Raji Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x 25 ml Ham's F12 Medium 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) 2 x 1 vial aAPC/Raji Cells	

Bio-Glo™ Reagents				
Bio-Glo™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo™ Luciferase Assay Buffer 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 1 x 1 vial Bio-Glo™ Luciferase Assay Substrate	1000
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer 1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate	100
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7940	1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer 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.

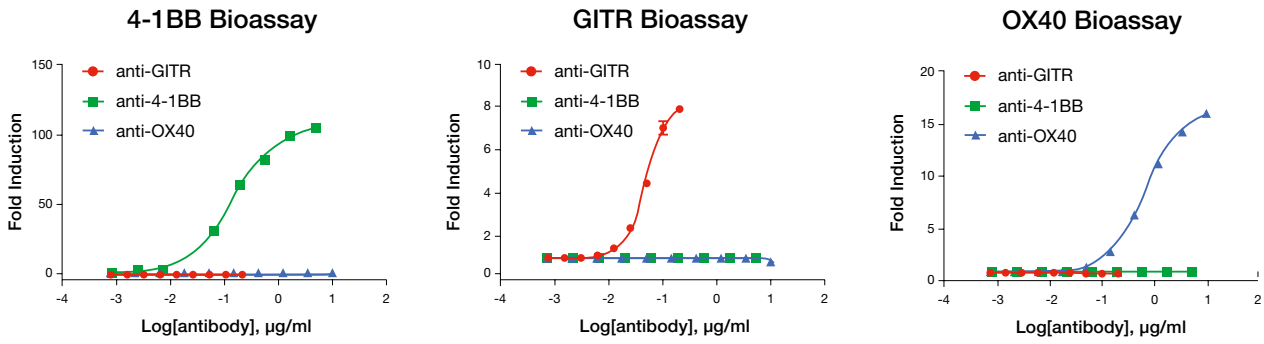
Co-Stimulatory Receptor Bioassays



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

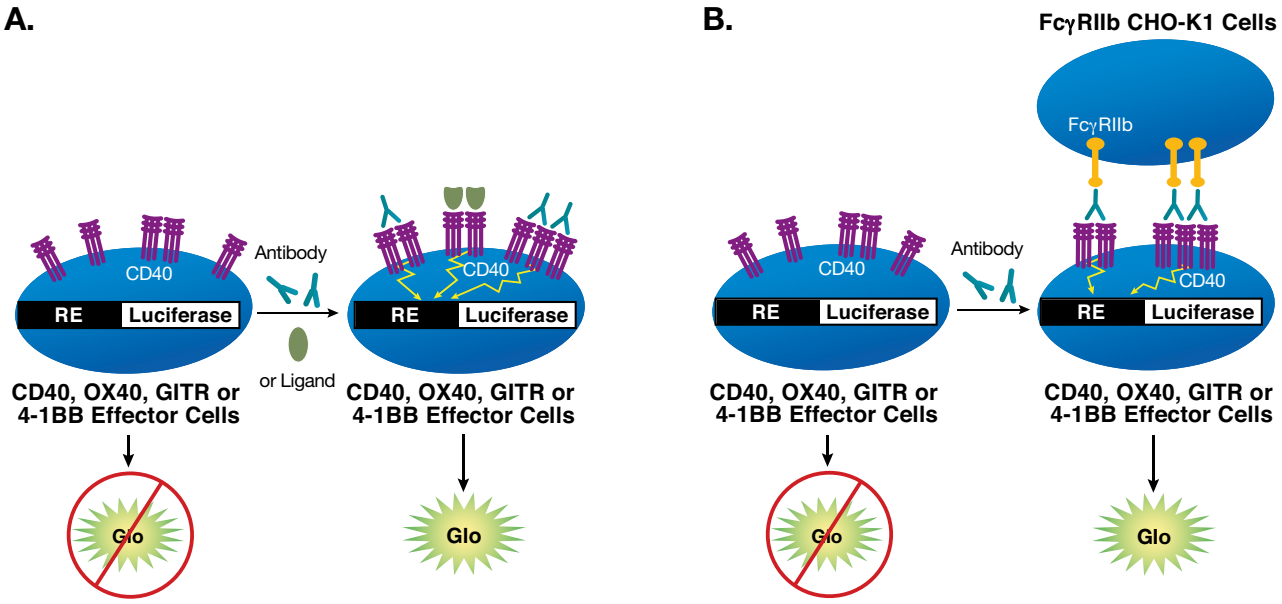
Co-stimulatory bioassay principle. The bioassay typically consists of an effector cell that expresses the co-stimulatory receptor and a luciferase reporter gene driven by upstream activation of the co-stimulatory receptor.

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
GITR Bioassay				
GITR Bioassay Kit	Catalog	JA2291	1 x 1 vial GITR Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
GITR Bioassay Kit 5X	Catalog	JA2295	5 x 1 vial GITR Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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
4-1BB (CD137) Bioassay				
4-1BB Bioassay Kit	Catalog	JA2351	1 x 1 vial 4-1BB Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
4-1BB Bioassay Kit 5X	Catalog	JA2355	5 x 1 vial 4-1BB Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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
OX40 Bioassay				
OX40 Bioassay Kit	Catalog	JA2191	1 x 1 vial OX40 Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
OX40 Bioassay Kit 5X	Catalog	JA2195	5 x 1 vial OX40 Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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
CD40 Bioassay				
CD40 Bioassay Kit	Catalog	JA2151	1 x 1 vial CD40 Effector Cells (U2OS) 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
CD40 Bioassay Kit 5X	Catalog	JA2155	5 x 1 vial CD40 Effector Cells (U2OS) 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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)	

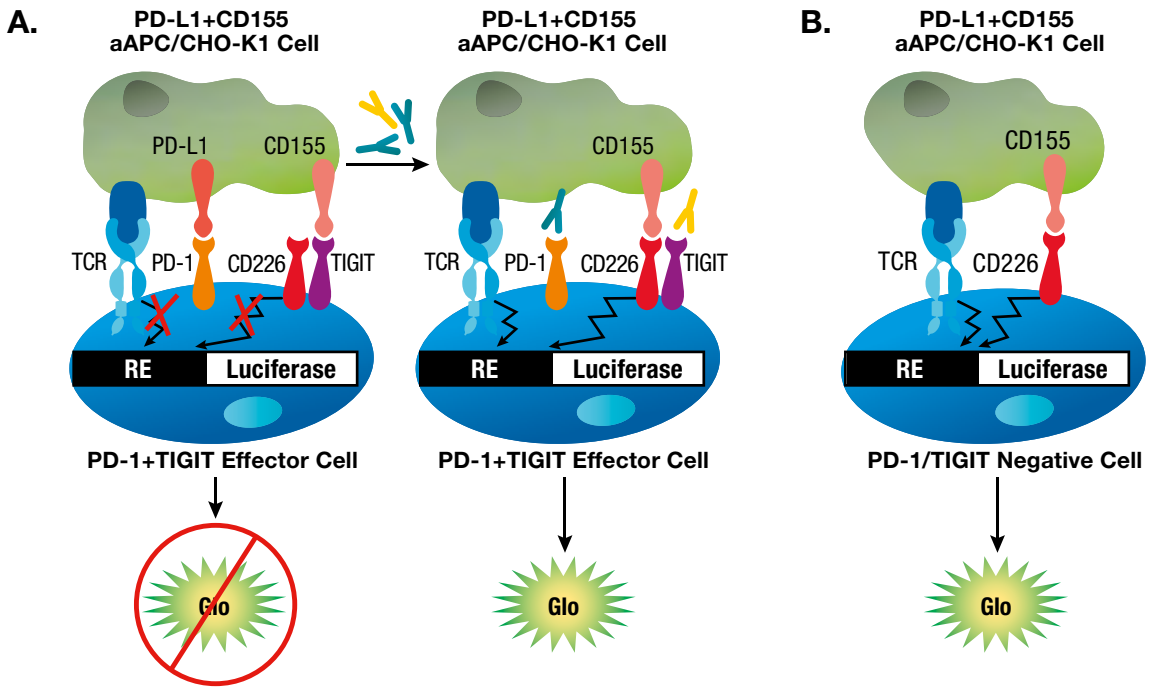
Co-Stimulatory Receptor Bioassays	Product Category	Cat. No.	Components	Assays in 96-well format
FcγRIIb/CHO-K1 Cells *Optimized for use in combination with the GITR, 4-1BB, OX40, and CD40 Effector Cells*				
FcγRIIb/CHO-K1 Cell Kit	Catalog	JA2251	1 x 1 vial FcγRIIb/CHO-K1 Cells 1 x 36 ml RPMI 1640 Medium	120
FcγRIIb/CHO-K1 Cells Kit 5X	Catalog	JA2255	5 x 1 vial FcγRIIb/CHO-K1 Cells 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 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
HVEM/LIGHT Bioassay Kit 5X	CAS	CS1979A32	5 x 1 vial HVEM Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
CD27 Bioassay Kit 5X	CAS	CS1979A25	5 x 1 vial CD27 Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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
DR3 Bioassay				
DR3 Bioassay Kit	CAS	CS1979E03	1 x 1 vial DR3 Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
DR3 Bioassay Kit 5X	CAS	CS1979E06	5 x 1 vial DR3 Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
DR3 Bioassay, Propagation Model	CAS	CS1979E02	2 x 1 vial DR3 Effector Cells	
ICOS Bioassay (Agonist format) *(utilizes NanoLuc® Luciferase)*				
ICOS Bioassay, Core Kit	CAS	CS1979D15	1 x 1 vial ICOS Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 36ml RPMI 1640 Medium 1 x Bio-Glo-NL™ Luciferase Assay System	120
ICOS Bioassay, Core Kit 5X	CAS	CS1979D18	5 x 1 vial ICOS Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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) **+** 2 x 1 vial FcγRIIb aAPC/CHO-K1 Cells (CS1979D04) **OR** 2 x 1 vial aAPC/CHO-K1 Cells (CS187110)	
CD28 Bioassay (Agonist format)				
CD28 Bioassay, Core Kit	CAS	CS1979C06	1 x 1 vial TCR/CD3 Effector Cells (IL-2) 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x 25 ml Ham's F12 Medium 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) 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 1 x 25 ml Ham's F12 Medium 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) **+** 2 x 1 vial FcγRIIb aAPC/CHO-K1 Cells (CPM) (CS1979D04) **OR** 2 x 1 vial aAPC/CHO-K1 Cells (CPM) (CS187110)	

Co-Stimulatory Receptor Bioassays	Product Category	Cat. No.	Components	Assays in 96-well format
FcγRIIb aAPC/CHO-K1 Cells *Optimized for use in combination with Effector Cells in the ICOS and CD28 bioassays for direct agonist measurement*				
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	
PD-L1 Negative Cells (aAPC/CHO-K1 Cells) *Optimized for use in combination with Effector Cells in the ICOS and CD28 bioassays for crosslinking-dependent agonist measurement*				
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	
Bio-Glo™ Reagents				
Bio-Glo™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo™ Luciferase Assay Buffer 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 1 x 1 vial Bio-Glo™ Luciferase Assay Substrate	1000
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer 1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate	100
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7940	1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer 1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate	1000

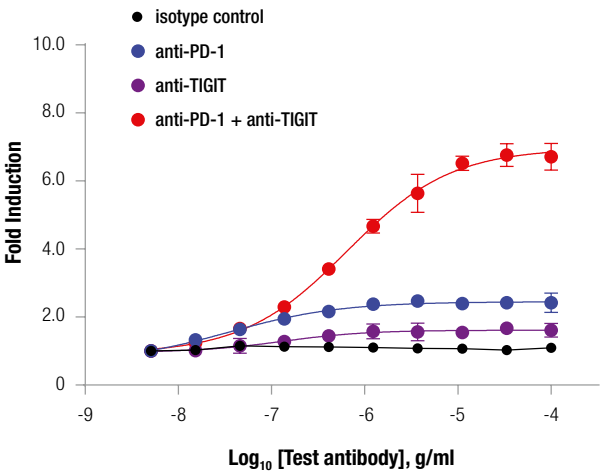
Note: Promega is constantly developing new bioassay formats, therefore please contact a Promega representative if you can't find your desired target in the ordering information.

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 1 x 1 vial PD-L1+CD155 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x Ham's F12 Medium 1 x 36 ml RPMI 1640 Medium 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 5 x 1 vial PD-L1+CD155 aAPC/CHO-K1 Cells 5 x 4 ml Fetal Bovine Serum 1 x Ham's F12 Medium 5 x 36 ml RPMI 1640 Medium 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 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 1 x 1 vial PD-L1+MHCII APC Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x 36 ml DMEM Medium 1 x 0.006 mg TCR Activating Antigen 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 5 x 1 vial PD-L1+MHCII APC Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x 36 ml DMEM Medium 5 x 0.006 mg TCR Activating Antigen 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 2 x 1 vial PD-L1 Raji Cells	

PD-1+4-1BB Combination Bioassay *(utilizes NanoLuc®luciferase)*				
PD-1+4-1BB Combination Bioassay Kit	CAS	CS1978I05	1 x 1 vial PD-1+4-1BB Effector Cells 1 x 1 vial PD-L1 aAPC/CHO-K1 Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 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 5 x 1 vial PD-L1 aAPC/CHO-K1 Cells 5 x 4 ml Fetal Bovine Serum 5 x 36ml RPMI 1640 Medium 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 2 x 1 vial PD-L1 aAPC/CHO-K1 Cells	

PD-1+CTLA-4 Combination Bioassay *(utilizes NanoLuc®luciferase)*	Product Category	Cat. No.	Components	Assays in 96-well format
PD-1+CTLA-4 Combination Bioassay Kit	CAS	CS1978D04	1 x 1 vial PD-1+CTLA-4 Effector Cells 1 x 1 vial PD-L1 aAPC/Raji Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 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 5 x 1 vial PD-L1 aAPC/Raji Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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 2 x 1 vial PD-L1 aAPC/Raji Cells	

Bio-Glo™ Reagents				
Bio-Glo™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo™ Luciferase Assay Buffer 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 1 x 1 vial Bio-Glo™ Luciferase Assay Substrate	1000
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7941	1 x 10 ml Bio-Glo-NL™ Luciferase Assay Buffer 1 x 1 vial Bio-Glo-NL™ Luciferase Assay Substrate	100
Bio-Glo-NL™ Luciferase Assay System	Catalog	G7940	1 x 100 ml Bio-Glo-NL™ Luciferase Assay Buffer 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.

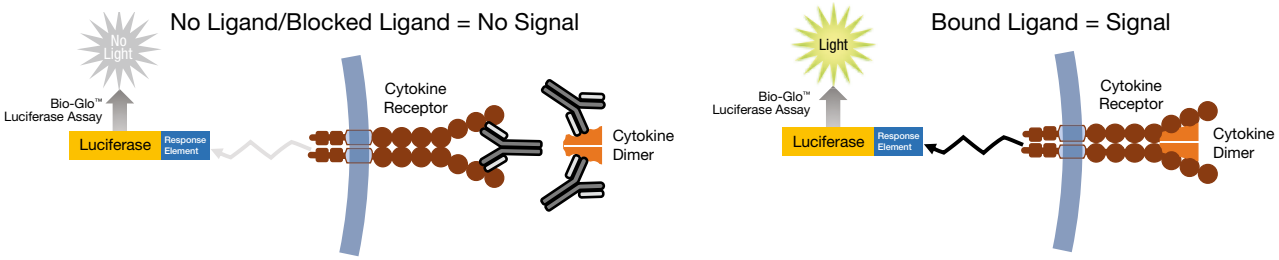
Cytokine and Growth Factor Bioassays

Benefits

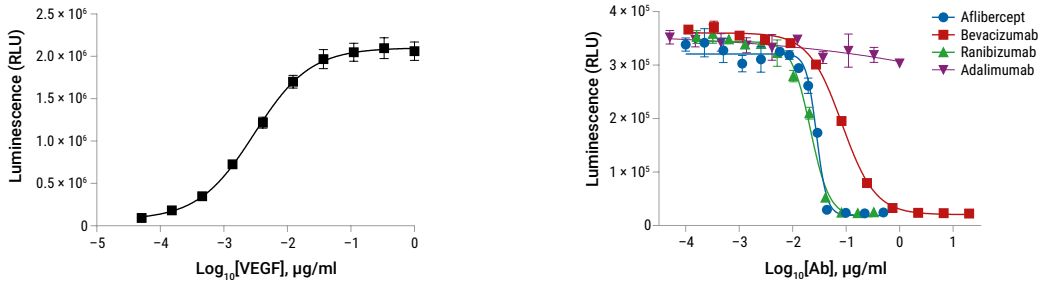
- Accelerate development of biosimilar drugs for cytokines and cytokine blockers
- Measure potency and manufacturing consistency of growth factor biosimilars and biobetters

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, TNFα, TGF-β, IFNβ and IFNγ 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 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
TNFα Bioassays				
TNFα Blockade Reporter Bioassay	CAS	CS177503	TNFα Receptor Cells (HEK293)	120
TNFα Reporter Cell Line	Catalog	E8520	2 x 1 vial NFkB HEK293 Cells	
TNFα Blockade Apoptosis Bioassay Designed for use with Promega's Caspase-Glo 3/7 Assay Reagents (Cat#G8091)	CAS	CS1324A05	TNFα Receptor Cells (U937)	120
Membrane TNFα Bioassay 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 Cross-listed in the Fc Effector chapter under anti-TNFα ADCC Bioassay	CAS	CS185501	Membrane TNFα Cells (CHO-K1)	
VEGF Bioassay				
VEGF Bioassay Kit	Catalog	GA2001	1 x 1 vial KDR HEK293 Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 60 ml DMEM 1 x Bio-Glo™ Luciferase Assay System	120
VEGF Bioassay Kit, 5X	Catalog	GA2005	5 x 1 vial KDR HEK293 Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 60 ml DMEM 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)	
RANKL Bioassay				
RANKL Bioassay Kit	CAS	CS2018D03	2 x 1 vial RANK Effector Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
RANKL Bioassay Kit 5X	CAS	CS2018D06	10 x 1 vial RANK Effector Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
RANKL Bioassay, Propagation Model	CAS	CS2018D01	2 x 1 vial RANK/NFkB U2OS Cells	
IL-1 Bioassay				
IL-1 Bioassay, Propagation Model	CAS	CS200401	2 x 1 vial hIL-8 A549 Cells	
IL-2 Bioassay				
IL-2 Bioassay Kit	Catalog	JA2201	1 x 1 vial IL-2 Bioassay Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
IL-2 Bioassay Kit 5X	Catalog	JA2205	5 x 1 vial IL-2 Bioassay Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 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
IL-6 Bioassay				
IL-6 Bioassay Kit	CAS	CS2018A18	1 x 1 vial SIE HEK293 Cells including other components	120
IL-6 Bioassay Kit 5X	CAS	CS2018A20	5 x 1 vial SIE HEK293 Cells including other components	600
IL-6 Bioassay, Propagation Model	CAS	CS187201	2 x 1 vial SIE HEK293 Cells	
IL-12 Bioassay				
IL-12 Bioassay Kit	CAS	CS2018A06	1 x 1 vial IL12R/STAT4-RE HEK293 Cells including other components	120
IL-12 Bioassay Kit 5X	CAS	CS2018A08	5 x 1 vial IL12R/STAT4-RE HEK293 Cells	600
IL-12 Bioassay, Propagation Model	CAS	CS2018A02	2 x 1 vial IL12R/STAT4-RE HEK293 Cells	
IL-15 Bioassay				
IL-15 Bioassay Kit	Catalog	JA2011	1 x 1 vial IL-15 Bioassay Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo™ Luciferase Assay System	120
IL-15 Bioassay Kit 5X	Catalog	JA2015	5 x 1 vial IL-15 Bioassay Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo™ Luciferase Assay System	600
IL-15 Bioassay, Propagation Model	Catalog	J2962	2 x 1 vial STAT5-RE CTLL-2 Cells	
IL-17 Bioassay *(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	
IL-23 Bioassay				
IL-23 Bioassay Kit	CAS	CS2018A10	1 x 1 vial IL-23R/IL-12R/SIE HEK293 Cells including other components	120
IL-23 Bioassay Kit 5X	CAS	CS2018A12	5 x 1 vial IL-23R/IL-12R/SIE HEK293 Cells including other components	600
IL-23 Bioassay, Propagation Model	CAS	CS2018A01	2 x 1 vial IL-23R/IL-12R/SIE HEK293 Cells	

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

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

For product information, visit:
www.promega.com/cas

To learn more about Promega bioassays for biologics, visit:

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

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

Or find your local Sales Support here:
www.promega.com/support

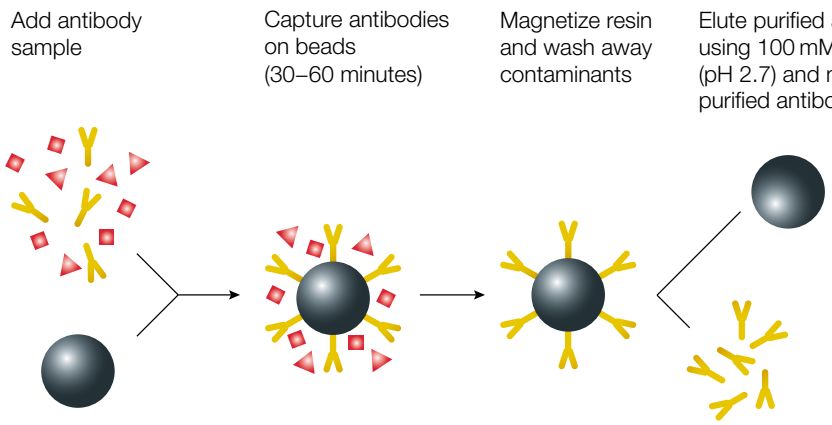
Antibody Purification

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.

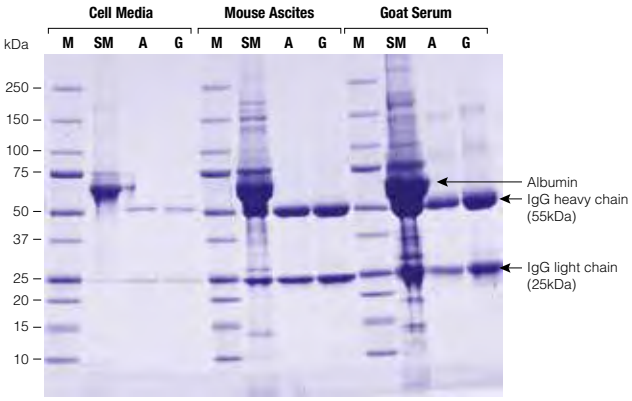
Optimized performance

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 50µl of cell culture media (mouse IgG1), mouse ascites (IgG2a) and goat serum with 50µl 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 5µl of purified sample (A or G) and stained with Coomassie®-based stain.

Ordering information

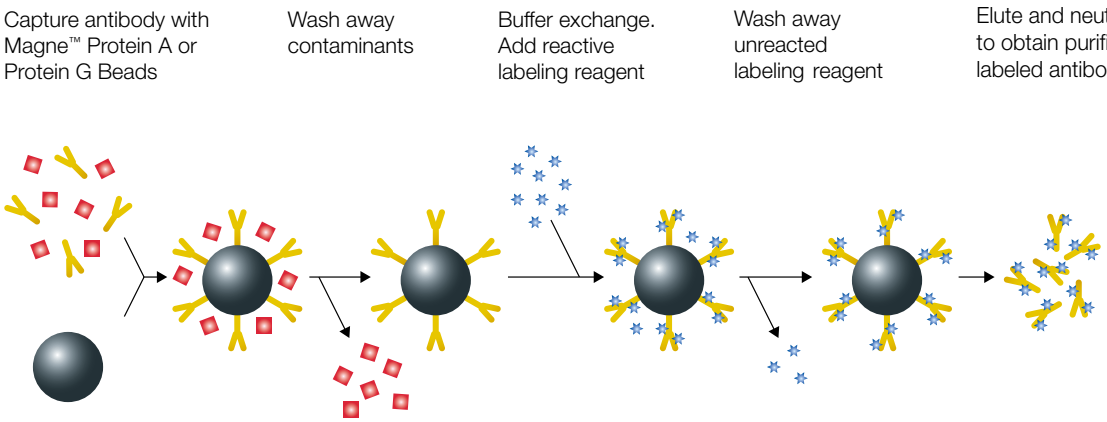
Product	Size	Cat. No.
Magne™ Protein G Beads, 20% Slurry	1 ml	G7471
Magne™ Protein G Beads, 20% Slurry	5 ml (5 × 1 ml)	G7472
Magne™ Protein G Beads, 20% Slurry	50 ml	G7473
Magne™ Protein A Beads, 20% Slurry	1 ml	G8781
Magne™ Protein A Beads, 20% Slurry	5 ml (5 × 1 ml)	G8782
Magne™ Protein A Beads, 20% 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



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

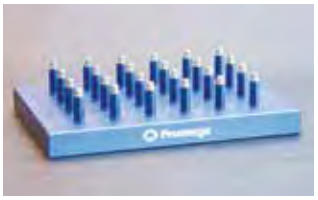
MagneSphere® Technology Magnetic Separation Stands

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

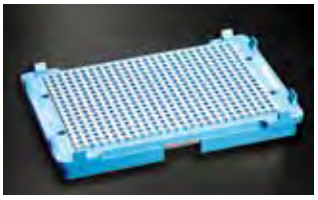
PolyATract® System 1000 Magnetic Separation Stand. One sample volume (1 – 50 ml) Cat. No. Z5410

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')$_2$ 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-termimal pyroglutamate and oxidation.

Features & benefits

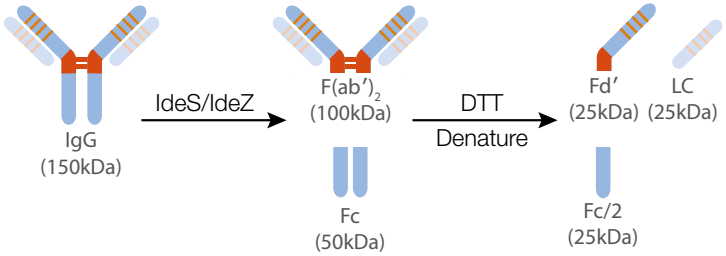
Fast and easy: Digestion in 30 minutes with no optimization

Highly specific and reproducible: Cleaves exclusively at a single site below the hinge to produce F(ab')$_2$ 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 (5 × 5000 units)	lyophilized	V7515
IdeZ Protease	5.000 units	lyophilized	V8341
IdeZ Protease	25.000 units (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

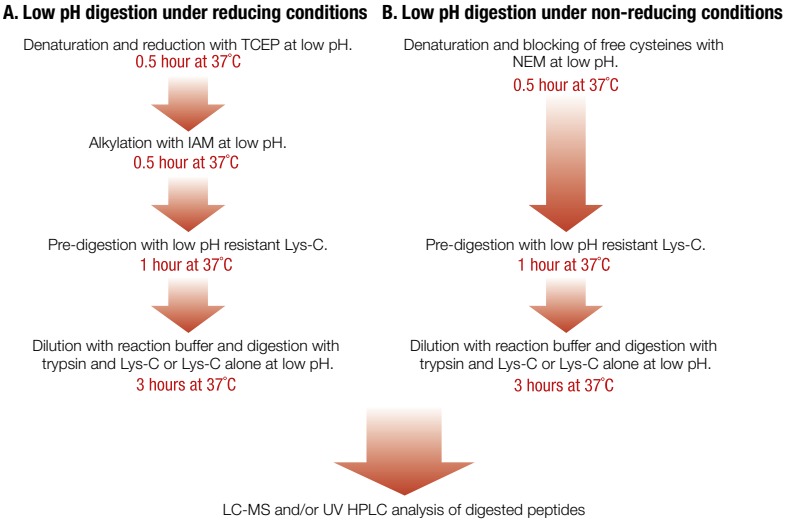
AccuMAP™ Low pH Protein Digestion Kit

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



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.

Worlwide Promega Contact Details

North America

United States/Canada
Tel: 608-274-4330
Fax: 608-277-2516
Toll-Free Tel: 800 356 9526
Toll-Free Fax: 800 356 1970
E-mail: custserv@promega.com

South America

Brazil
Tel: +55 11 5090 3780
Fax: +55 11 5096 3780
E-mail: promega.brasil@promega.com

Australia

Australia
Tel: 02 8338 3800
Fax: 02 8338 3855
Freecall: 1800 225123
Freefax: 1800 626 017
E-mail: auscustserv@promega.com

Asia

China
Tel: +86 10 5825 6268
Fax: +86 10 5825 6160
Toll-Free: 800 810 8133
E-mail: info@promega.com.cn

Japan
Tel: 03 3669 7981
Fax: 03 3669 7982
E-mail: jpmktg@jp.promega.com

Korea
Tel: +82 2158 83718
Fax: +82 2262 85418
E-mail:
CustServiceKR@promega.com

Pacific Asia Region, Singapore
Tel: +65 6513 3450
Fax: +65 6773 5210
E-mail: sg_custserv@promega.com

India
Tel: +91 11 43005814/15/16/17
Fax: +91 11 41035028
E-mail: ind_custserv@promega.com

Europe

Germany/Austria
Tel: +49 6227 6906-291
Fax: +49 6227 6906-222
E-mail: de_custserv@promega.com

Switzerland
Tel: +44 878 90 00
Fax: +44 878 90 10
E-mail: ch_custserv@promega.com

France
Tel: +33 0437 2250 00
Fax: +33 0437 2250 10
Numero Vert: 0 800 48 79 99
E-mail: contactfr@promega.com

Italy
Tel: +39 0254 0501 94
Fax: +39 0256 5616 45
Toll-Free Phone: 800 6918 18
E-mail:
customerservice.italia@promega.com

Poland
Tel: +48 22 531 0667
Fax: +48 22 531 0669
E-mail: pl_custserv@promega.com

**Belgium/Luxembourg/
The Netherlands**
Tel: +31 71 532 42 44
Fax: +31 71 532 49 07
E-mail: benelux@promega.com

Spain
Tel: +34 902 538 200
Fax:+34 902 538 300
E-mail: esp_custserv@promega.com

**Denmark, Estonia, Finland,
Iceland, Norway, Sweden**
Tel: +46 8 452 2450
Fax: +46 8 452 2455
E-mail: sweorder@promega.com

United Kingdom
Tel: +44 23 8076 0225
Fax: +44 23 8076 7014
Free Phone: 0800 378994
E-mail: ukcustserve@promega.com

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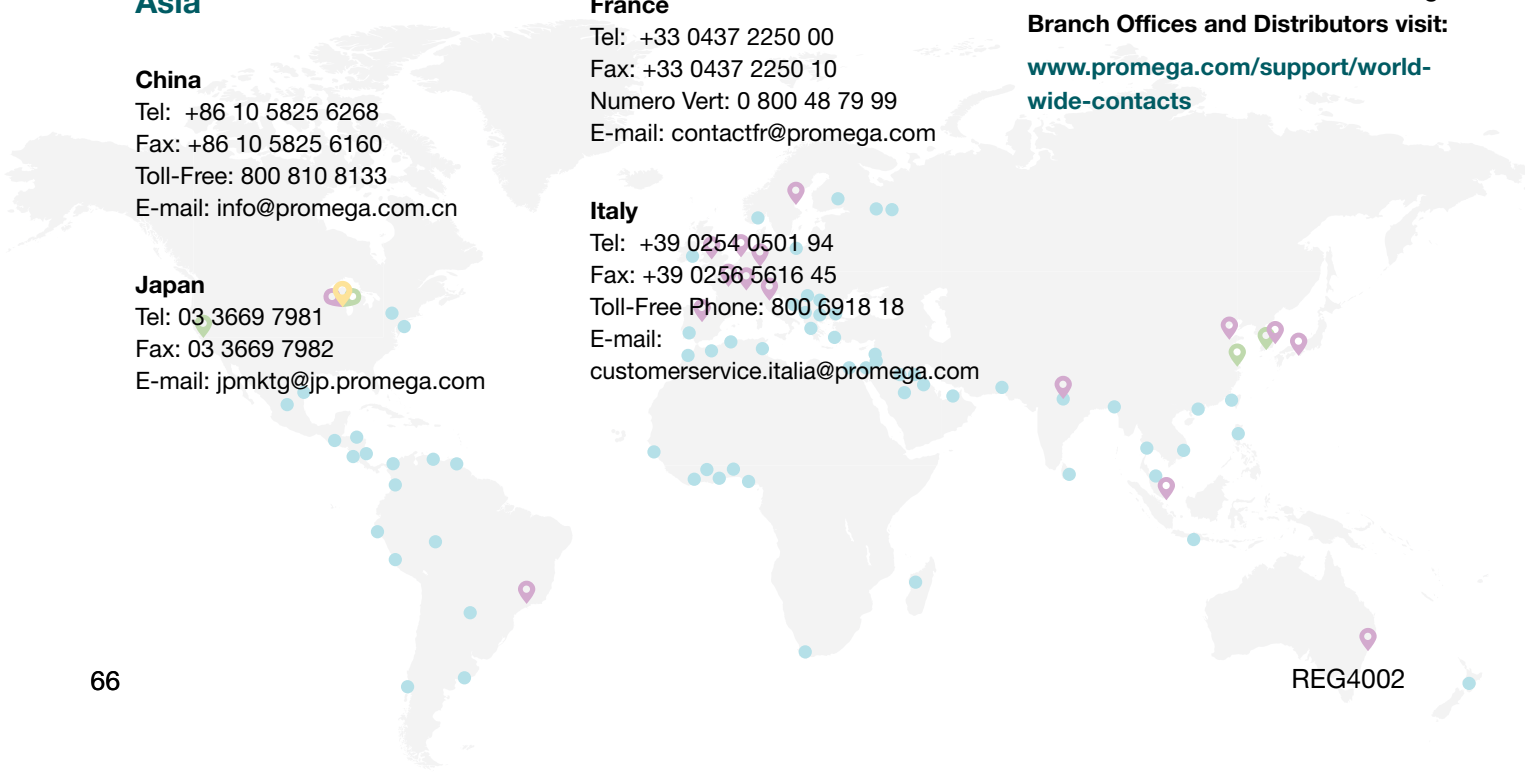
www.promega.com/support/world-wide-contacts



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