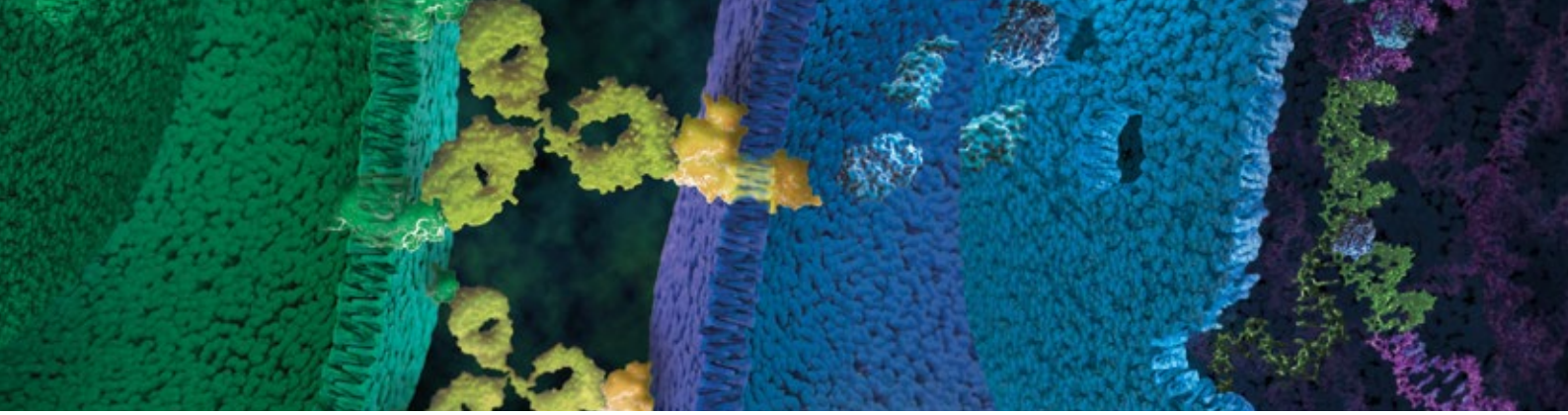
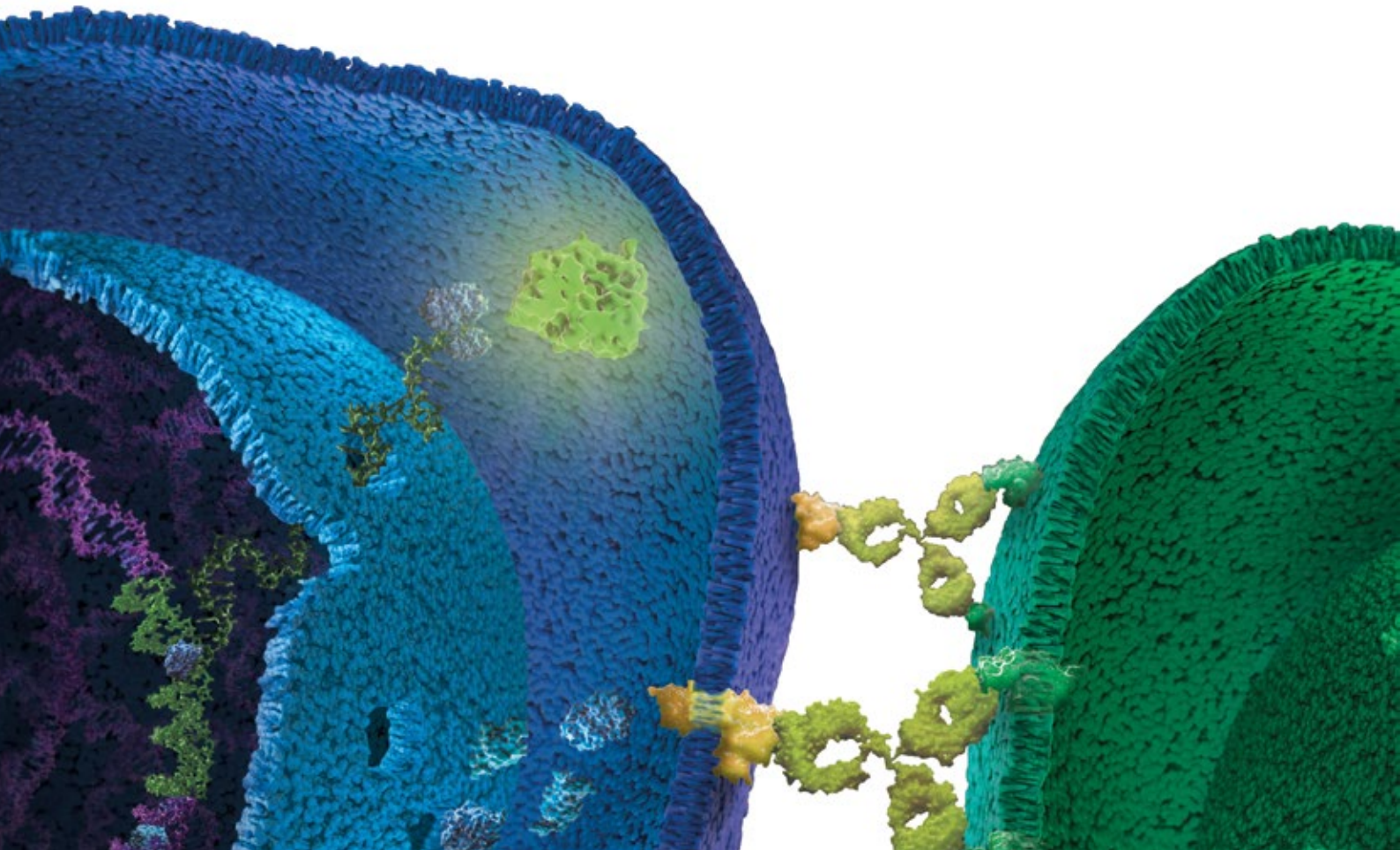
Promega

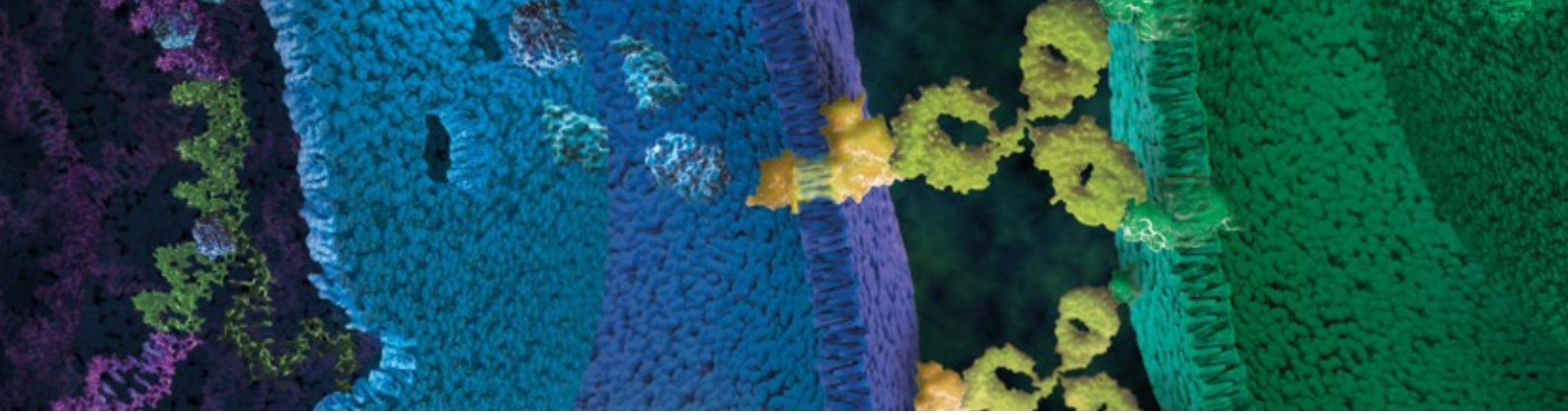
Tools for the Development of Functional Bioassays



Promega's Bioassays for FC Effector Function and Immunotherapy

The Mechanism of action-based bioassays are precise, accurate and have a low variability. The cells are available in a "ready-to-use" frozen format.





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Signaling Pathway Based Bioassays for Biologics

Tools for the Development of Functional Bioassays for Potency Evaluation, QC and Lot Release Assay Development

Development of antibody and non-antibody based biologics requires functional bioassays for potency evaluation, QC and lot release. For characterization of therapeutic antibodies and a variety of non-antibody biologics either "on the market" or in clinical development, Promega has developed various signaling pathway based reporter assays for a wide range of targets. These assays represent an applicable basis for functional bioassays. All assays 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 warrantys.

General bioassay fundamentals

Simple and robust detection

The majority of Promega's assay cell lines have been developed by using the firefly luciferase reporter 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 reporter signal is achieved by measuring luminescence using Promega's Bio-Glo™ Luciferase Assay System.

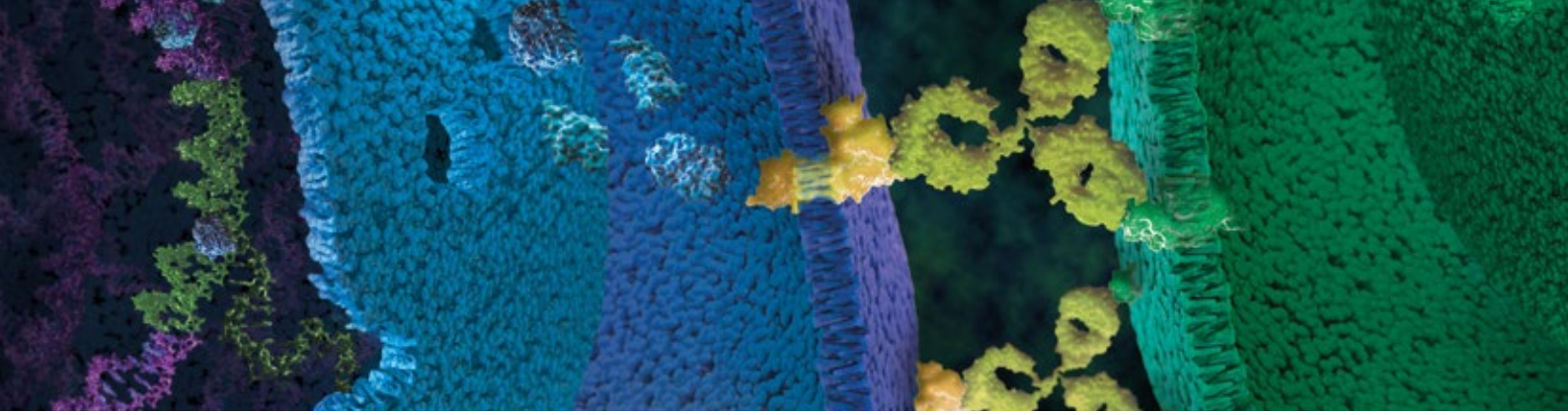
Convenient "thaw-and-use" cells

"Thaw-and-use" or propagation in continuous culture

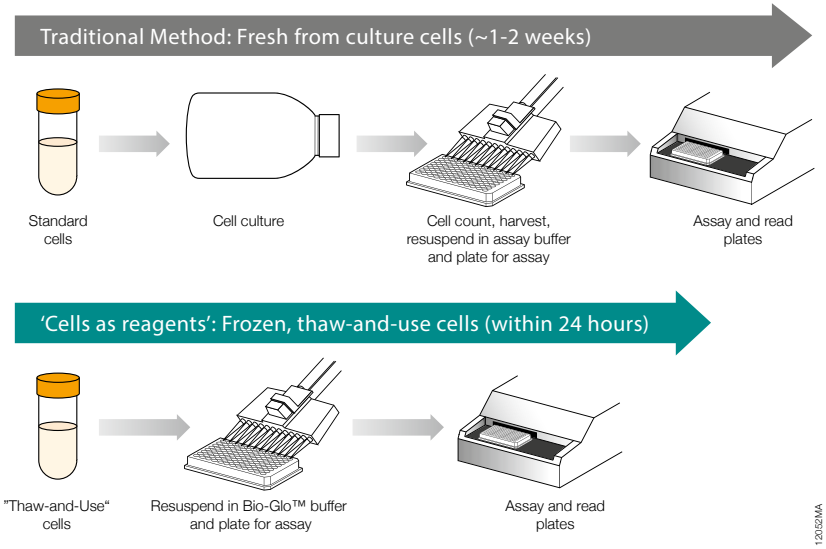
All bioassay cell lines are available in "thaw-and-use" format. Cells provided as "thaw-and-use" have been optimized for direct use in the assays without need for continuous cell culture. This greatly minimizes assay variability and simplifies experiment planning and logistics. Complete kits contain all of the components and reagents (frozen "thaw-and-use" cells along with additional materials like reagents, target cells, etc) necessary to perform an anti-CD20-based ADCC Reporter Bioassay. The kit provides a control anti-CD20 antibody and is an ideal way for a new user to become acquainted with the bioassay.

Propagation model

As an alternative to thaw-and use cells, cell lines for continous propagation in culture allow researchers to create and manage their own master and working cell banks for future use. This so called "Cell Propagation Model" (CPM) is sold as a tool for assay development and characterization of biologics in an R&D environment for drug discovery, development and monatoring of biologics, vaccines and product release. For other usage please do not hesitate to contact your local Promega representative.



“Thaw-and-use” formats for rapid assay development



Bio-Glo™ Luciferase Assay System

Bio-Glo™ is a highly sensitive, robust and homogeneous reagent for the detection of firefly luciferase reporter gene expression in cell-based bioassays. The reagent is more stable and has an improved tolerance to sample components than standard luciferase assays. Bio-Glo™ Assay reagent is functionally tested for performance and is intended for use in Promega’s Reporter Bioassays.

- **Simplified assay optimisation:** Robust performance, improved storage and convenient size
- **Room temperature / 4°C storage:** Extended stability of the Bio-Glo™ Assay reagent makes it more convenient for everyday use
- **Improved assay precision:** Less sensitive to mixing and dispensing conditions, enhancing reproducibility and making it ideal for bioassay applications
- **Extended bright light output:** High sensitivity, especially for extended incubations such as 24 hours, optimized for batch and continuous-process handling
- **Reduced unwanted effects from sample components:** Bio-Glo™ Assay is less sensitive to culture media, phenol red and luciferase inhibitors compared to other luciferase assays

Ordering information

Product	Size	Cat. No.
Bio-Glo™ Luciferase Assay System	100ml	G7940
Bio-Glo™ Luciferase Assay System	10ml	G7941

Fc Effector Bioassays for ADCC and ADCP Analysis

Bioluminescent Cell-Based Bioassays for Therapeutic Antibody Development

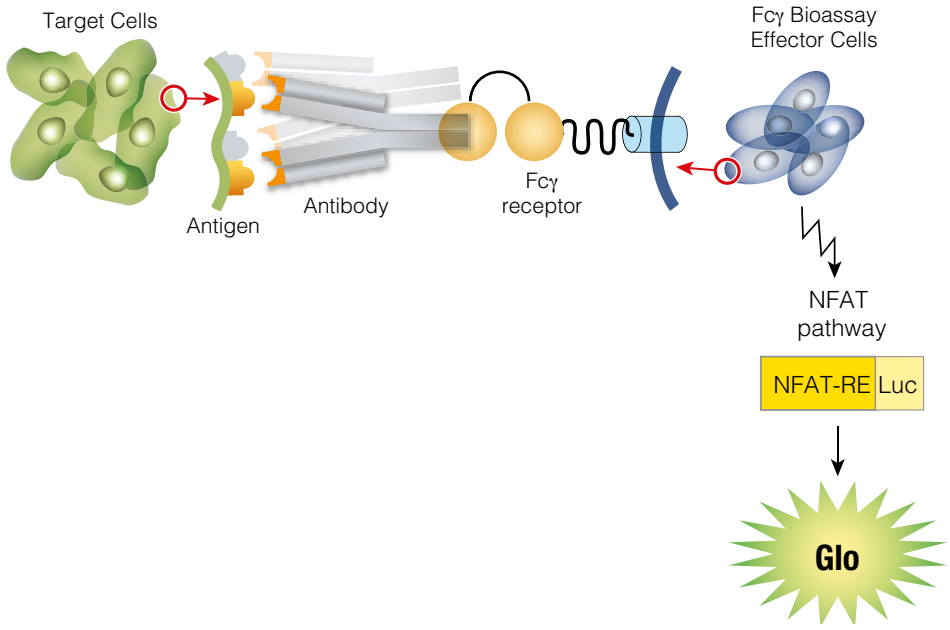
Drug developers and regulatory authorities recognize antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) as important mechanism of action (MOA) of therapeutic antibodies. Traditional ADCC and ADCP Bioassays use primary cells, which are labor intensive and highly variable. However, less easy-to-use and consistent analysis of these important MOA is needed in drug development programs. To meet this needs, we have developed a suite of highly specific, sensitive, accurate and reproducible cell-based Fc Effector Function Reporter Bioassays. The bioassays utilize a genetically engineered stable Jurkat Effector Cell to express a specific human 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 using the Bio-Glo™ Assay and a luminometer.

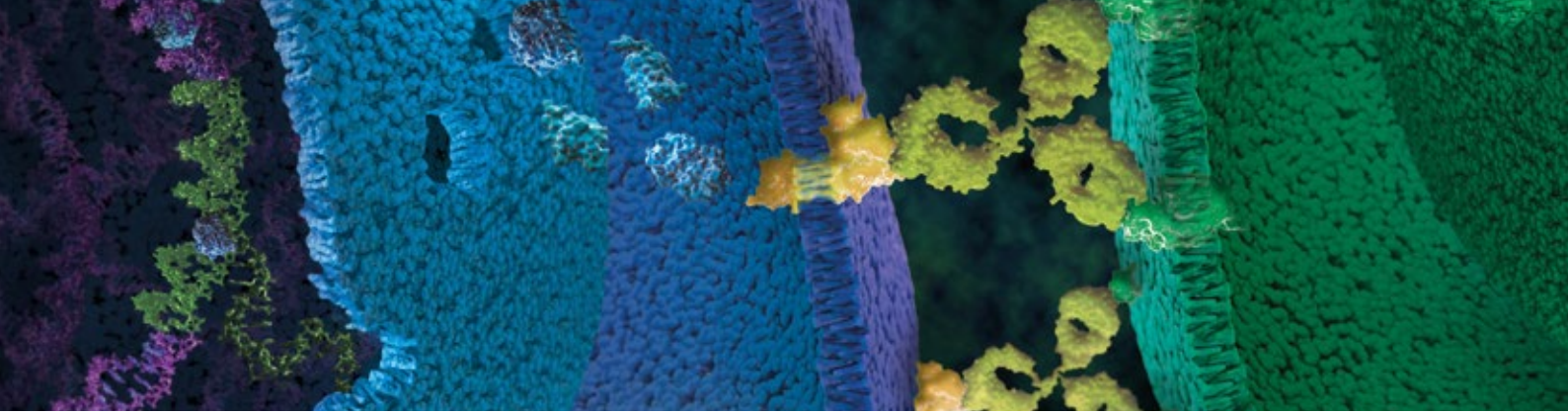
Fc Effector Function Reporter Bioassays have been developed to quantify antibody-mediated signaling through the following receptors:

- **Human FcγRIIIa (V158 and F158 variants)**
- **Human FcγRIIa (H131 variant; R131 variant)**
- **Human FcγRI**
- **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 and potency determination.

FC Reporter Bioassay principle

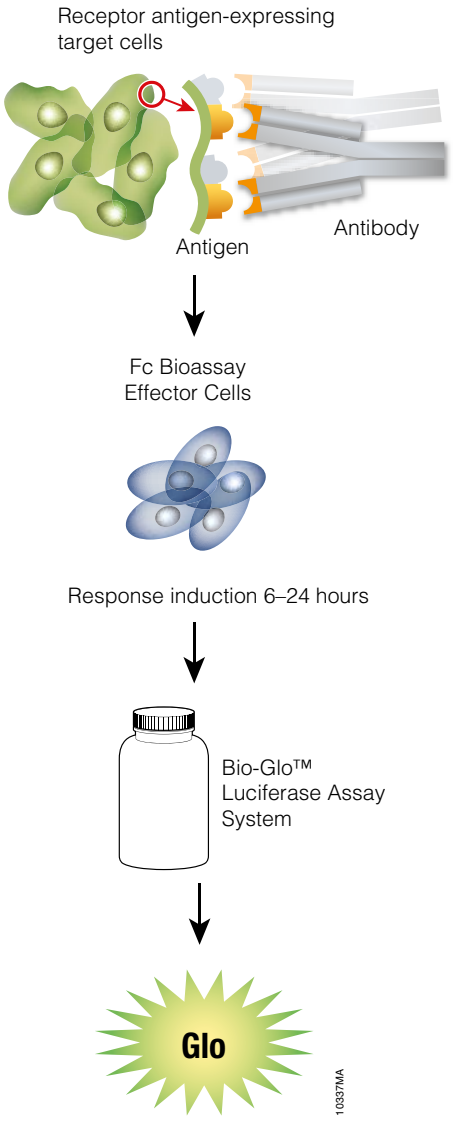




Fc Reporter Bioassay concept

Brief pre-incubation of antigen-presenting target cells with therapeutic antibody is followed by addition of the genetically engineered Effector Cells. Subsequent activation of the Fc receptor signalling 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 and 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).

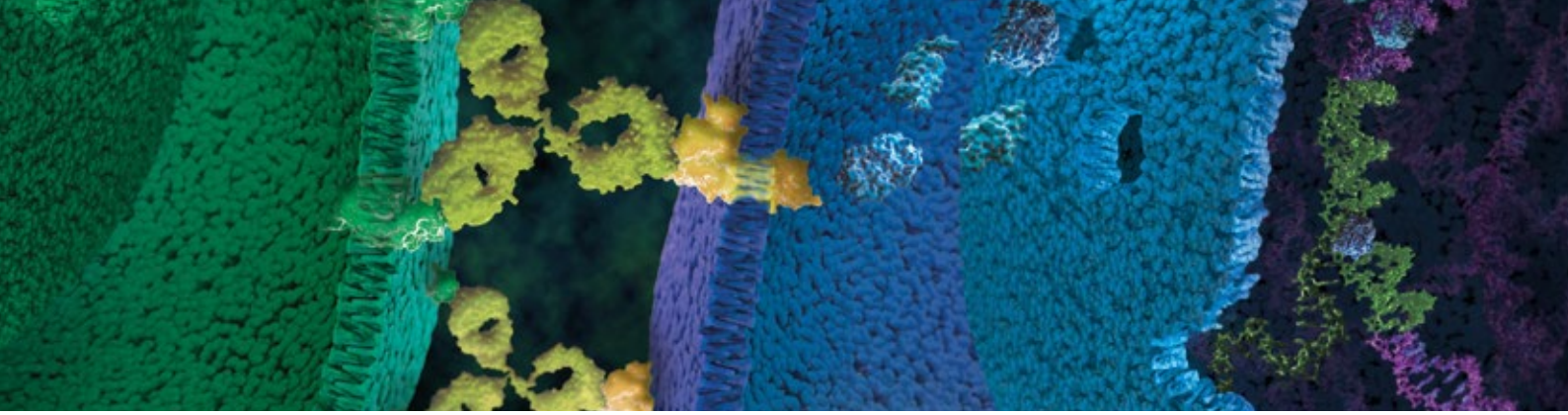
Schematic protocol for the Fc Reporter Bioassay



Results can be obtained in less than 1 working day with a simple, robust methodology

Suitable for Quality Control lot release

By eliminating 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

Traditional mechanism of action 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, 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 signalling pathway due to activation of the NFAT (Nuclear Factor of Activated T-Cells) response element.

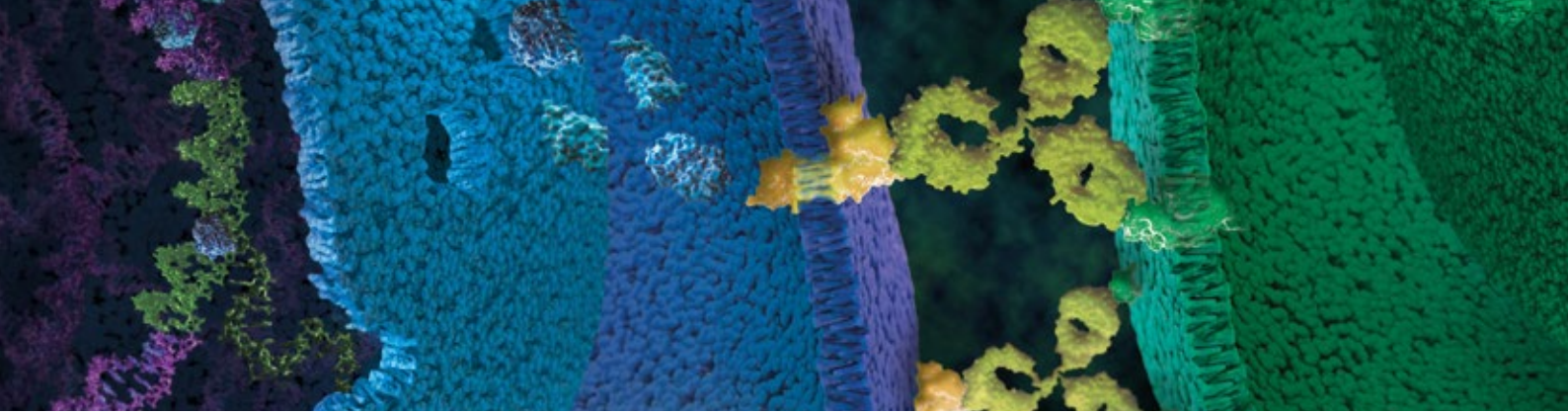
Ideal bioassay

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

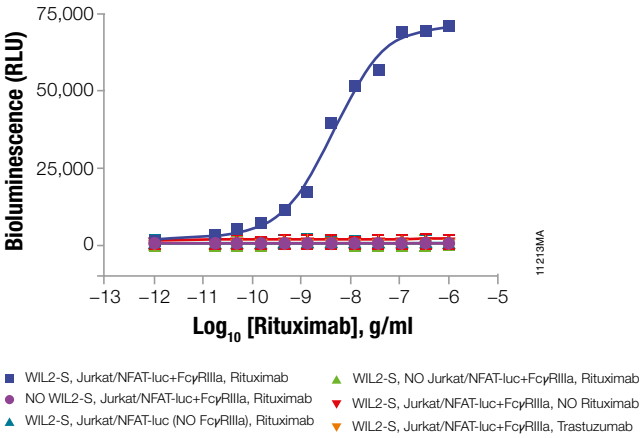
ADCC is a Fc effector function involving binding of antigen-bound antibody Fc domains with the FcγRIIIa receptor on immune system “killer” cells. Polymorphism in the FcγRIIIa receptor at amino-acid 158 results in both high affinity (V158) and low affinity (F158) variants FcγRIIIa genotypes (e.g. VV, 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



Specificity of the ADCC Reporter Bioassay

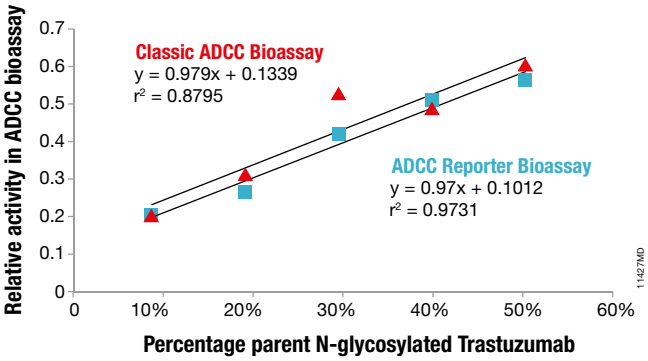
The ADCC Reporter Bioassay exhibits the clear specificity desired for a bioassay. The reporter gene luminescent response is only present when target cells with the correct surface antigen, the correct specific antibody and effector cells expressing FcαRIIIa are present. If any of these is missing, no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



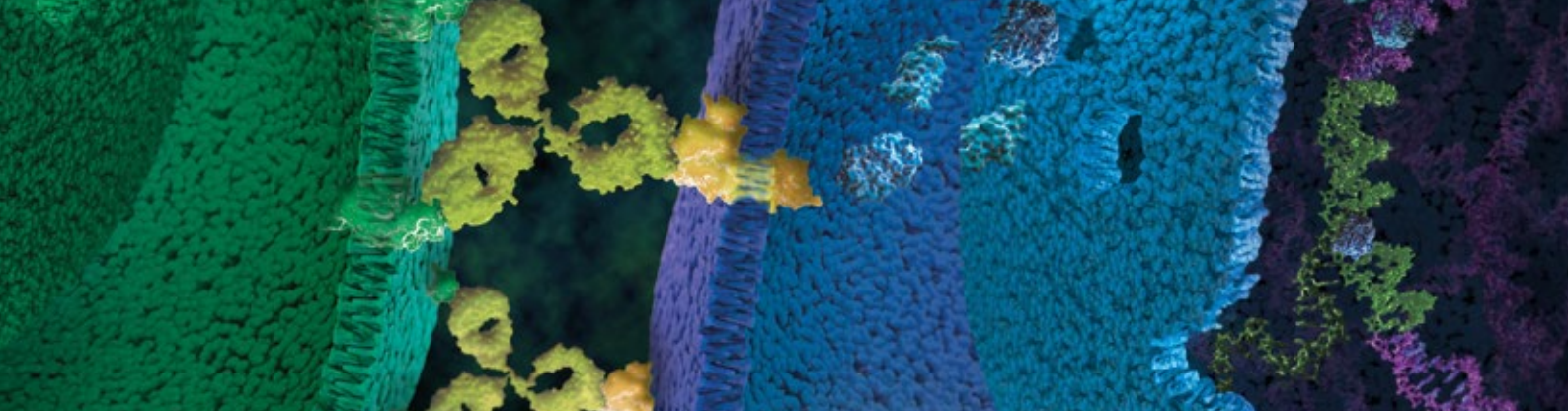
Serial dilutions of Rituximab (anti-CD20), Trastuzumab (anti-Her2) or assay medium control (no antibody) were incubated for 6 hrs at 37°C with engineered Jurkat Effector Cells (ADCC Bioassay Effector Cells) with or without ADCC Bioassay Target Cells (WIL2-S), as indicated. Luciferase activity was quantified using Bio-Glo™ Reagent. Data were fitted using 4PL curve fitting of GraphPad Prism® software.

Correlation with classic ADCC data

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

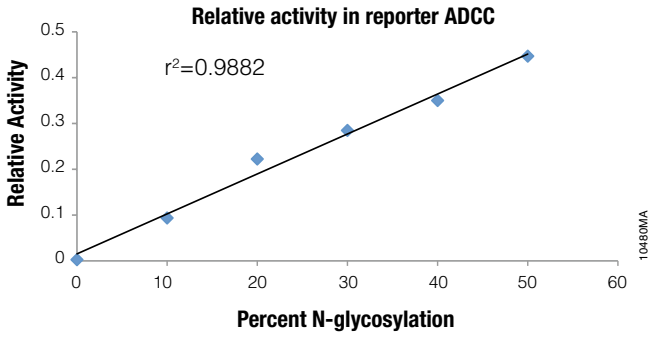


Correlation of relative ADCC activity with fraction of Trastuzumab N-glycosylation. Trastuzumab was N-deglycosylated using PNGase F, blended with fully N-glycosylated parent preparations to create test samples representing different % N-glycosylation (indicated on the X-axis) and assayed using either 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. For the ADCC Reporter Bioassay, ADCC pathway activation was measured by quantification of luciferase activity in the Effector Cell; for classic ADCC bioassay LDH release from target cells was measured. For both assays biological activity reflects downstream effects of Effector Cell FcγRIIIa crosslinking by antibody bound to target cells. Biological activity was determined and expressed relative to fully N-glycosylated Trastuzumab and plotted against percent N-glycosylated Trastuzumab.



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 highlighted that activity changes associated with small (5 – 10 %) changes in glycosylation level are easily detectable with the ADCC Reporter Bioassay.



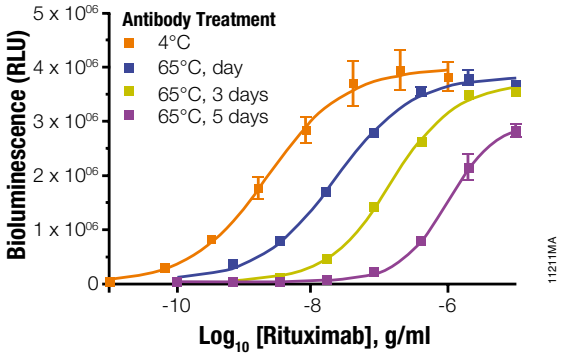
Antibody glycosylation: relative activity in ADCC Reporter Bioassay

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. Target cells were ADCC Bioassay Target Cells (WIL2-S) and the E:T ratio was 6:1. Biological activity was expressed relative to the 100 % control run in the same assay plate and plotted against the % of N-glycosylation present. Linear regression analysis was performed to determine correlation.

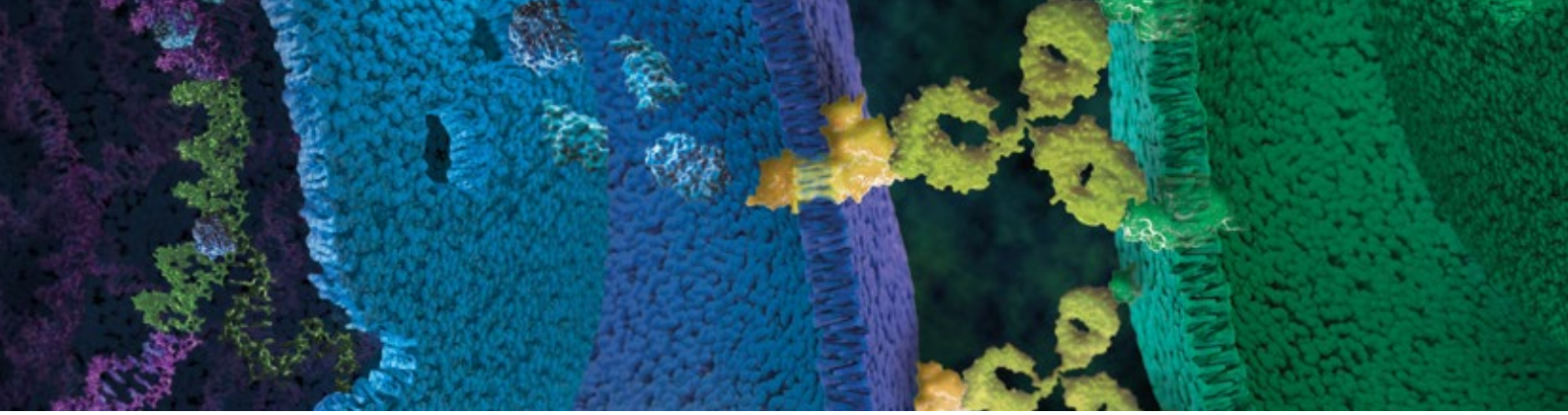
Stability indicating

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}



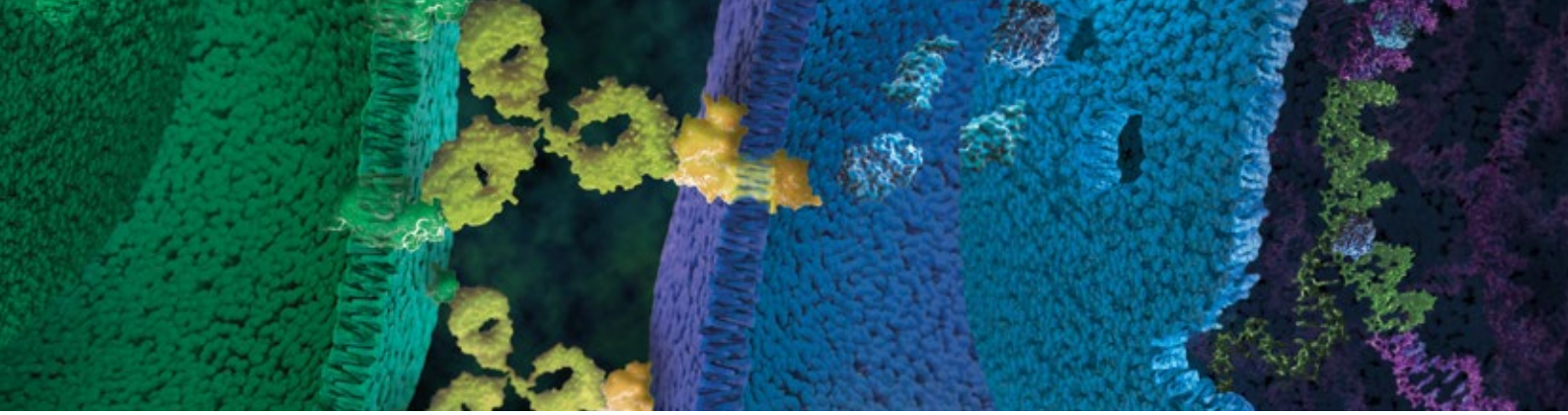
The data included here show the relative activity in the ADCC Reporter Bioassay of antibody stored at elevated temperature (65 °C) for various periods of time compared to a control sample stored at 4°C.



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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
ADCC Bioassay Effector Cells, Propagation Model	Catalog	G7102	2 x 1 vial ADCC Bioassay Effector Cells (2×10^7/ml)	No limit

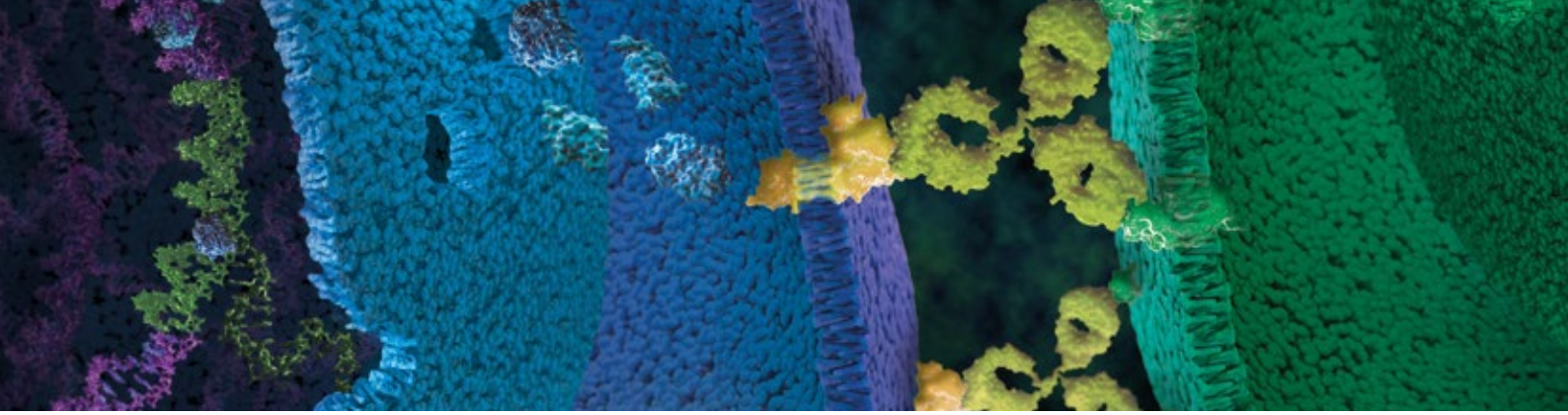
Human FcγRIIIa-F Variant ADCC				
ADCC Reporter Bioassay, F Variant, Core Kit	Catalog	G9790	1 x 1 vial ADCC Bioassay Effector Cells, F Variant 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	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)	No limit



FcγR Effector Bioassay Bundles Human FcγR Bioassay 5-Pack (includes catalog & CAS Effector Cells)	Product Category	Cat. No.	Components	Assays in 96-well format
Human FCγR Bioassay 5-Pack	CAS	Please inquire	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 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	120

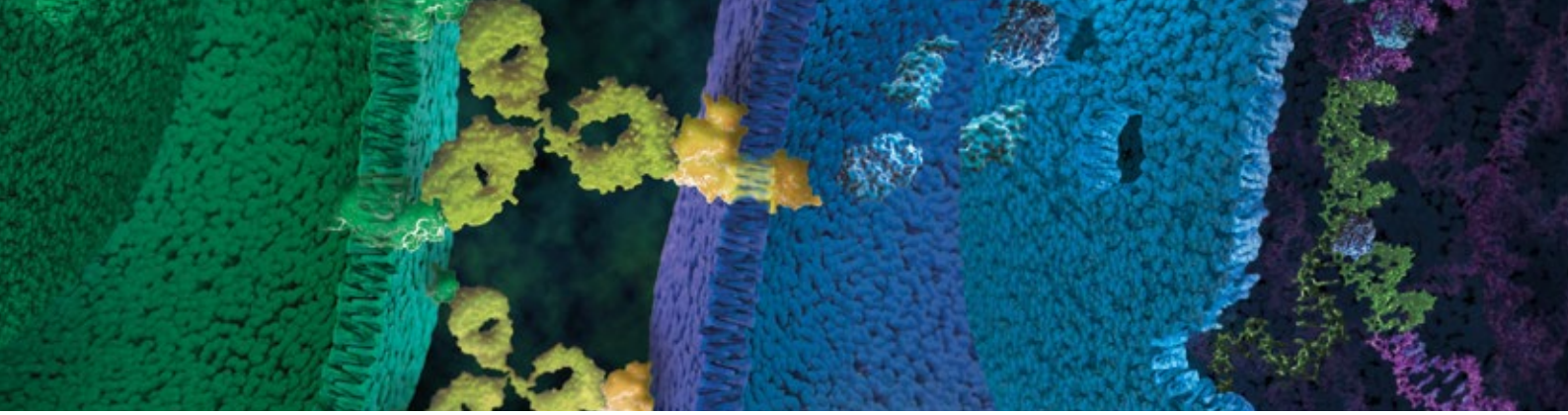
Mouse FcγRIV ADCC				
mFcγRIV ADCC Reporter Bioassay, Complete Kit	Catalog	M1201	1 x 1 vial mFcgRIV Effector Cells 1 x 1 vial Target Cells (Raji) 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate 1 x 1 vial Control Ab, Anti-CD20	120
mFcγRIV ADCC Reporter Bioassay, Core Kit	Catalog	M1211	1 x 1 vial mFcgRIV Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer	120
mFcgRIV 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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
mFcγRIV ADCC Bioassay Effector Cells, FcgRIV, Propagation Model	Catalog	M1212	2 x 1 vial mFcgRIV Effector Cells (2 x 10^7/ml)	No limit

Mouse FcγRIII ADCC				
mFcγRIII ADCC Reporter Bioassay, Core Kit	CAS	CS1779B08	1 x 1 vial mFcgRIII Effector Cells 1 x 4 ml Low IgG Serum 1 x 36 mL RMP 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
mFcγRIII ADCC Bioassay Effector Cells, Propagation Model	CAS	CS1779B06	2 x 1 vial mFcgRIII Effector Cells (2 x 10^7/ml)	No limit



Bio-Glo Reagents	Product Category	Cat. No.	Components	Assays in 96-well format
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	No limit



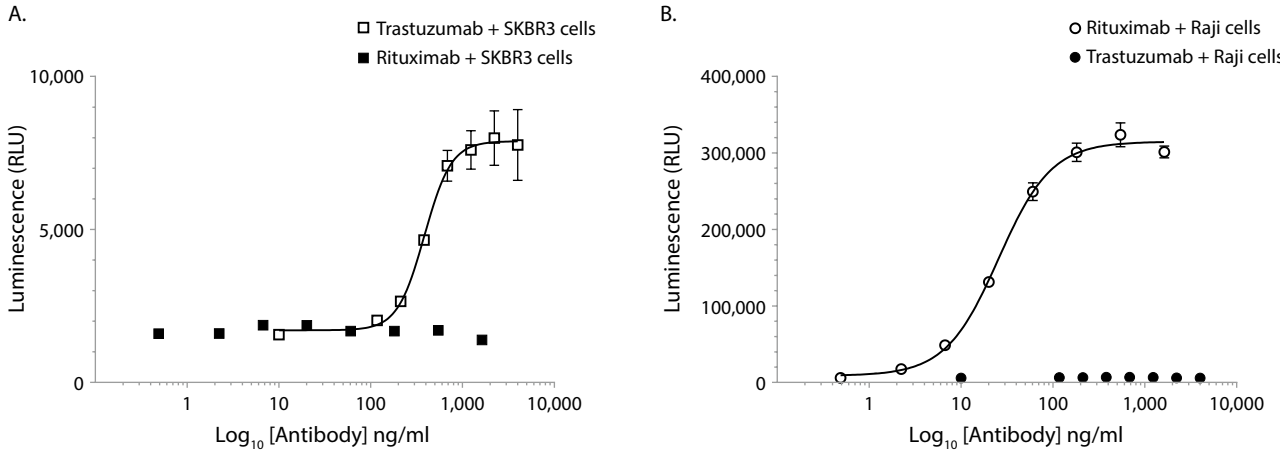
Antibody-Dependent Cell-Mediated Phagocytosis (ADCP) Assays

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).

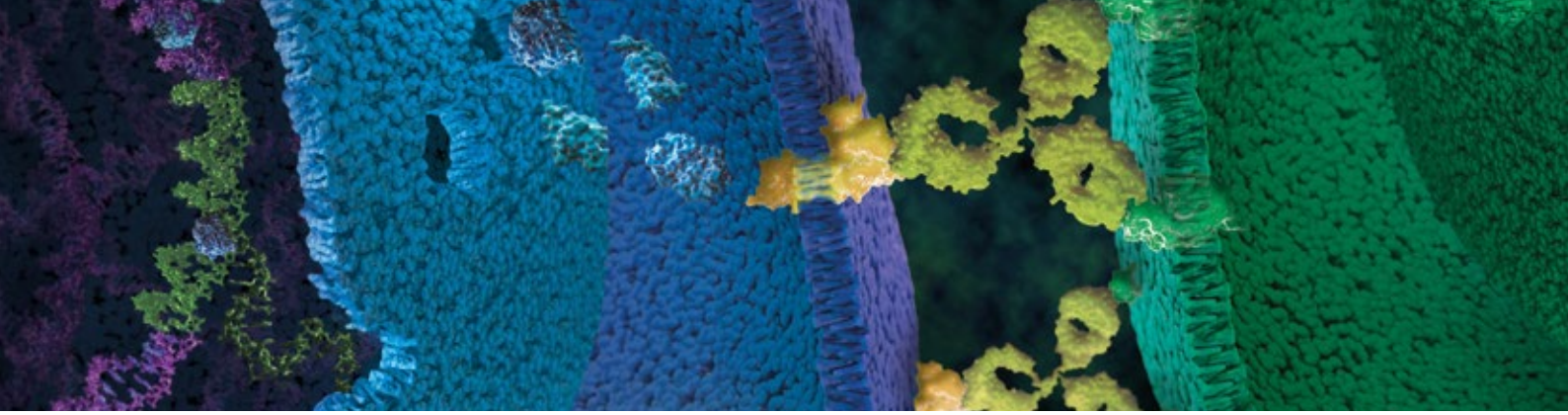
The FcγRIIa-H ADCP Reporter Bioassay is a bioluminescent cell-based assay that overcomes the limitations of existing assays. It can be used to measure the potency and stability of antibodies and other biologics with Fc domains that specifically bind and activate FcγRIIa. The assay consists of a genetically 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 without the need for propagation.

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 cell gives an assay response, whereas no response is obtained when antibody cannot bind to target cells.



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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
Human FcγRIIa-R Variant ADCP				
FcγRIIa-R ADCP Reporter Bioassay, Core Kit	CAS	CS1781B01-1	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
FcγRIIa-R ADCP Bioassay Effector Cells, Propagation Model	CAS	CS1781B06	2 x 1 vial FcγRIIa-R Bioassay Effector Cells (2×10^7/ml)	No limit
Human FcγRI Variant ADCP				
FcγRI ADCP Reporter Bioassay, Core Kit	CAS	CS1781C02-1	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
FcγRI ADCP Bioassay Effector Cells, Propagation Model	CAS	CS1781C06	2 x 1 vial FcγRI ADCP Bioassay (2×10^7/ml)	No limit
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

T Cell Activation Bioassays

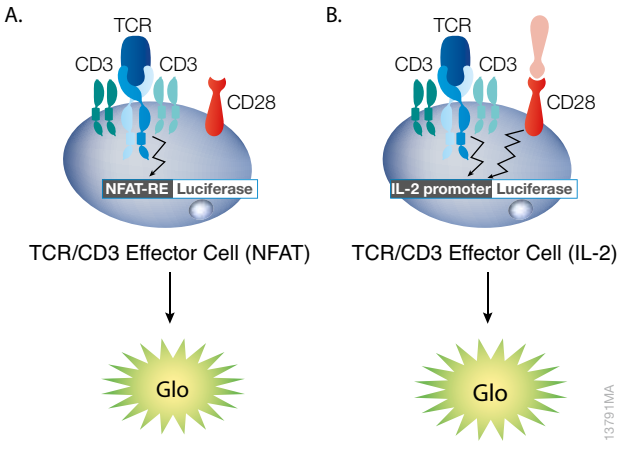
A Robust Reporter-Based T Cell Activation Assay for Biologics in Immunotherapy

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 T Cell Receptor (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 resulting in IL-2 production.

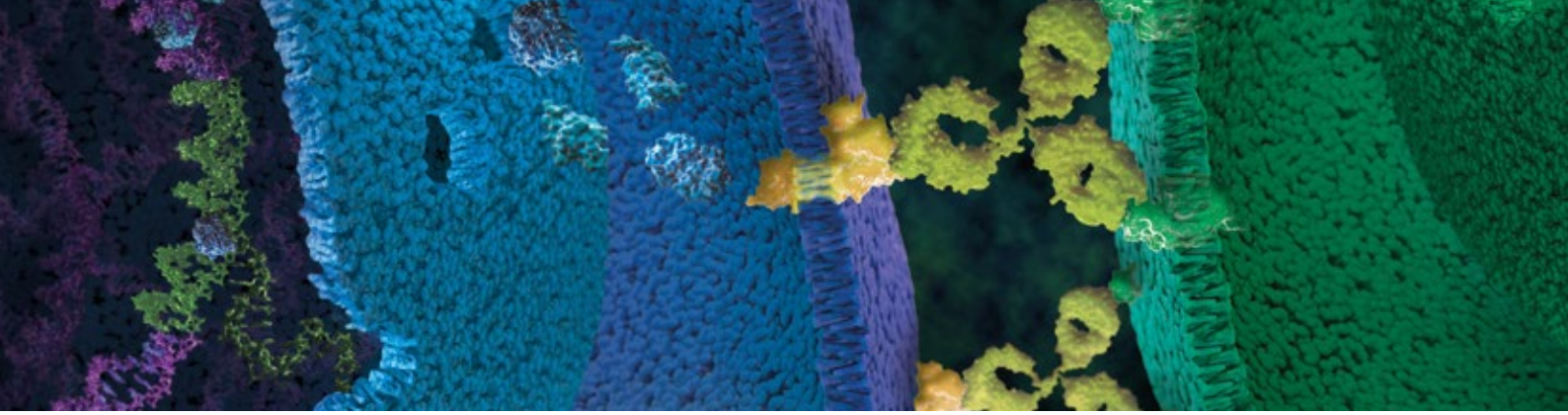
Promega's T Cell Activation Bioassays are bioluminescent cell-based assays that overcome the limitations of existing methods. The assays and can be used for the discovery and development of novel biologics such as bispecific antibodies and CAR-T cell therapies. The assays consist of a genetically engineered Jurkat T cell line (TCR/CD3 Effector Cells) that expresses a luciferase reporter 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. Both Effector Cells (NFAT as well as IL2 driven) are provided in a "thaw-and-use" format as cryopreserved cells that can be thawed, plated and used in an assay without the need for cell propagation.

The bioassay is prequalified according to ICH guidelines and shows the precision, accuracy and linearity required for routine use in potency and stability studies. The bioassay workflow is simple, robust and compatible with 96-well and 384-well plate formats used for antibody screening and drug discovery. Additionally, the bioassay is tolerant to human serum, indicating potential for further development into a neutralizing antibody bioassay.

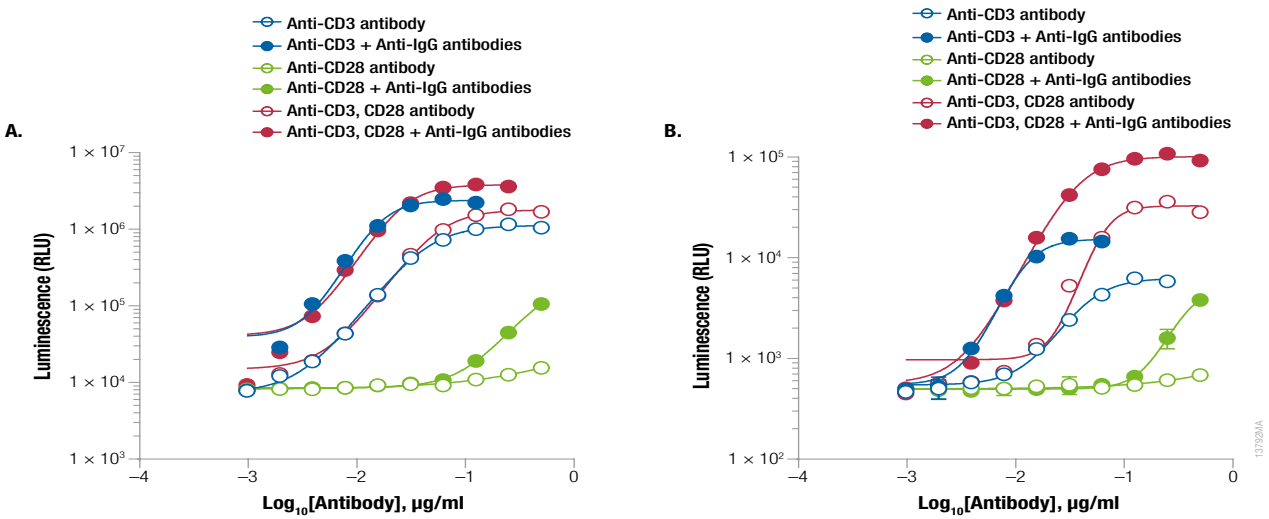
The T Cell Activation Bioassay principle



The T Cell Activation Bioassay (NFAT) and T Cell Activation Bioassay (IL-2) each consist of a genetically engineered cell line, TCR/CD3 Effector Cells (NFAT; Panel A) and TCR/CD3 Effector Cells (IL-2; Panel B), respectively. When engaged with either an anti-TCR/CD3 stimulus alone or an anti-TCR/CD3 and an anti-CD28 stimulus, receptor-mediated signaling induces luminescence (via activation of the NFAT or IL-2 pathway, respectively). Reporter gene activity can be detected by addition of Bio-Glo™ Reagent and quantitated with a GloMax® Detection System.

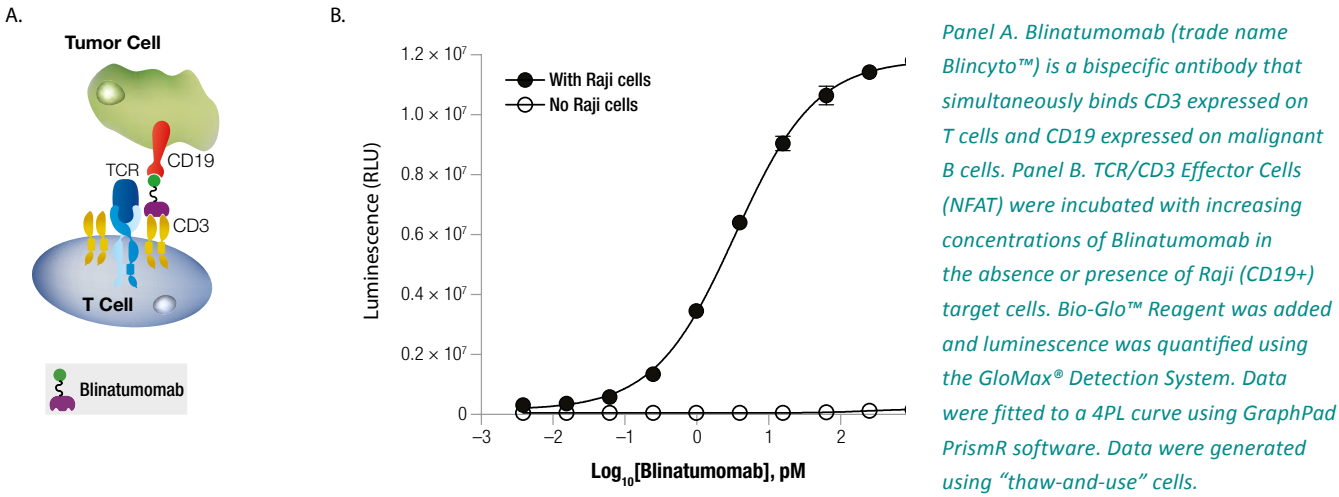


The T Cell Activation Bioassay reflects the MOA of biologics designed to engage the TCR and induce TCR-mediated T cell activation

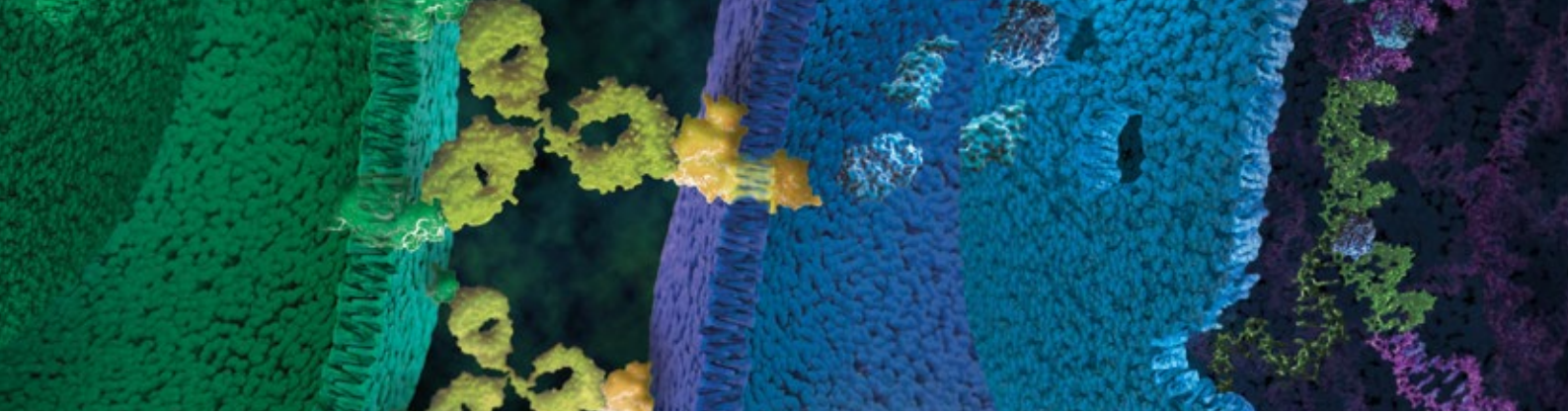


TCR/CD3 Effector Cells (NFAT; Panel A) or TCR/CD3 Effector Cells (IL-2; Panel B) were incubated with anti-CD3, anti-CD28 or both antibodies followed by either no crosslinking or crosslinking with a goat anti-mouse IgG antibody, as indicated. Bio-Glo™ Reagent was added and luminescence was quantified using the GloMax® Detection System. Data were fitted to a 4PL curve using GraphPad PrismR software. Data were generated using "thaw-and-use" cells.

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



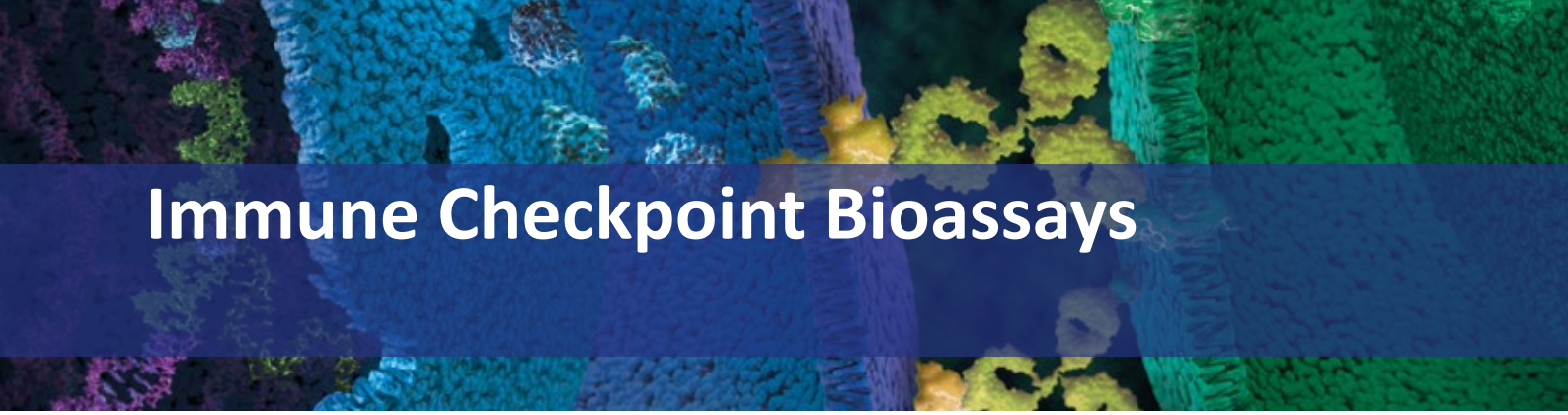
Panel A. Blinatumomab (trade name Blincyto™) is a bispecific antibody that simultaneously binds CD3 expressed on T cells and CD19 expressed on malignant B cells. Panel B. TCR/CD3 Effector Cells (NFAT) were incubated with increasing concentrations of Blinatumomab in the absence or presence of Raji (CD19+) target cells. Bio-Glo™ Reagent was added and luminescence was quantified using the GloMax® Detection System. Data were fitted to a 4PL curve using GraphPad PrismR software. Data were generated using "thaw-and-use" cells.



T Cell Activation Bioassay: 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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
T Cell Activation Bioassay (NFAT), Propagation Model	Catalog	J1601	2 x 1 vial TCR/CD3 Effector Cells (NFAT) (CPM)	No limit
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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
T Cell Activation Bioassay (IL-2), Propagation Model	Catalog	J1631	2 x 1 vial TCR/CD3 Effector Cells (NFAT) (CPM)	No limit

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



Immune Checkpoint Bioassays

Immune Checkpoint Bioassays to Characterize Immune-Modulating Antibodies

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 (e.g. PD-1, CTLA-4, TIGIT, LAG-3) and co-stimulatory (e.g. GITR, 4-1BB, OX40, CD40) 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.

Current methods used to measure the potency of these therapeutic drugs rely on binding assays or primary cell-based assays which are unable to provide a mechanism of action-based measure of drug potency with the precision and accuracy required for use in controlled drug development environment. Promega's reporter-based immune checkpoint bioassays provide a simple, consistent and reliable cell-based assay to measure antibody function throughout the drug research and development pipeline. For each target mentioned above, stable cell lines were generated which express an immune checkpoint receptor and a luciferase reporter driven by a response element specifically responding to signaling mediated by mostly TCR or directly from the immune receptor.

Furthermore, combination Bioassays are able to provide a quantitative measure of the synergistic effect of two immune checkpoint antibodies on T cell activation. 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.

Biologically relevant measurement of Ab MOA

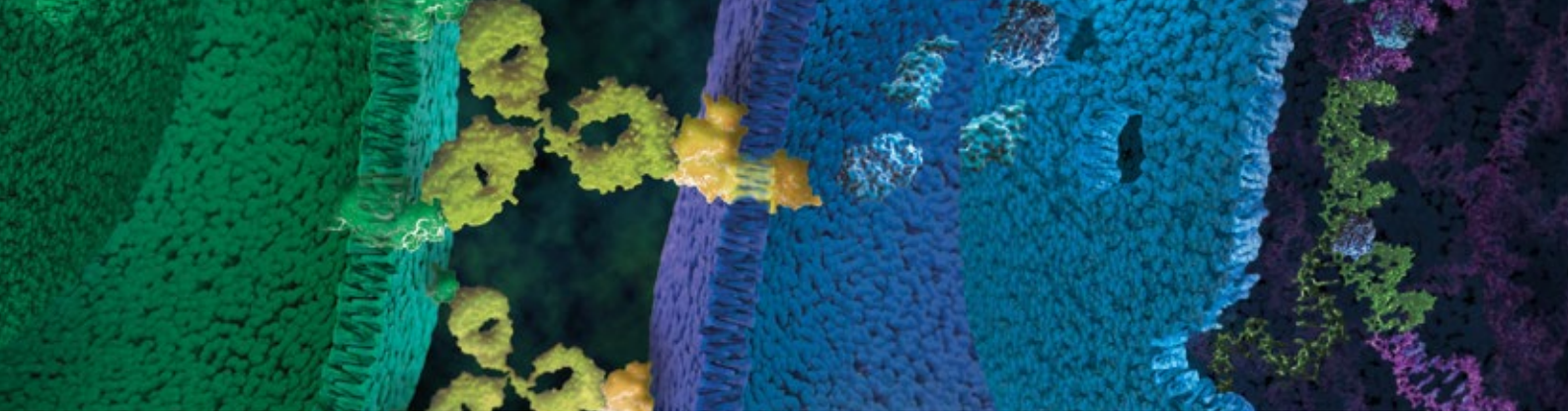
- Blockade or engagement of specific immune checkpoint pathways induces luciferase activity
- Well established and recognized reporter assay format
- Predictive of true biology

Consistent and reliable measure of Ab potency and stability

- Demonstrated precision, accuracy, reproducibility and robustness
- "Thaw-and-use" cell format, no cell culture required
- Bioassay kits include all required reagents in standardized formats

Easy to implement

- Convenient product formats allowing rapid and streamlined workflow
- Amenable to high-throughput formats



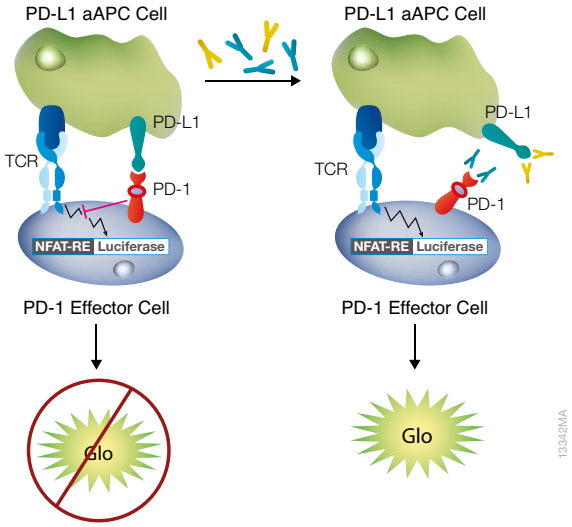
PD-1/PD-L1 Blockade Bioassay for Individual or Combination Immune Checkpoint Immunotherapy

The PD-1/PD-L1 Blockade Bioassay is a bioluminescent cell-based assay that can be used to measure the potency and stability of antibodies and other biologics designed to block the PD-1/PD-L1 interaction. The assay consists of two genetically engineered cell lines: PD-1 Effector Cells and PD-L1 aAPC*/CHO-K1 Target Cells. PD-1 Effector Cells are Jurkat T cells expressing human PD-1 and a luciferase reporter driven by a NFAT response element (NFAT-RE). The target cell line PD-L1 aAPC/CHO-K1 expresses human PD-L1 and a TCR activator (an engineered cell surface protein) designed to maximally activate TCRs in an antigen-independent manner.

The PD-1 Effector Cells and PD-L1 aAPC/CHO-K1 Cells are provided in "thaw-and-use" format as cryopreserved cells that can be thawed, plated and used in an assay without the need for cell propagation. During co-cultivation of the two cell lines the TCR becomes maximally activated by the TCR activator and at the same time the PD-1/PD-L1 interaction inhibits TCR signaling and NFAT-RE-mediated luminescence. Addition of either an anti-PD-1 or anti-PD-L1 antibody that blocks the PD-1/PD-L1 interaction releases the inhibitory signal and results in reconstituted TCR signaling and NFAT-RE-mediated luminescence.

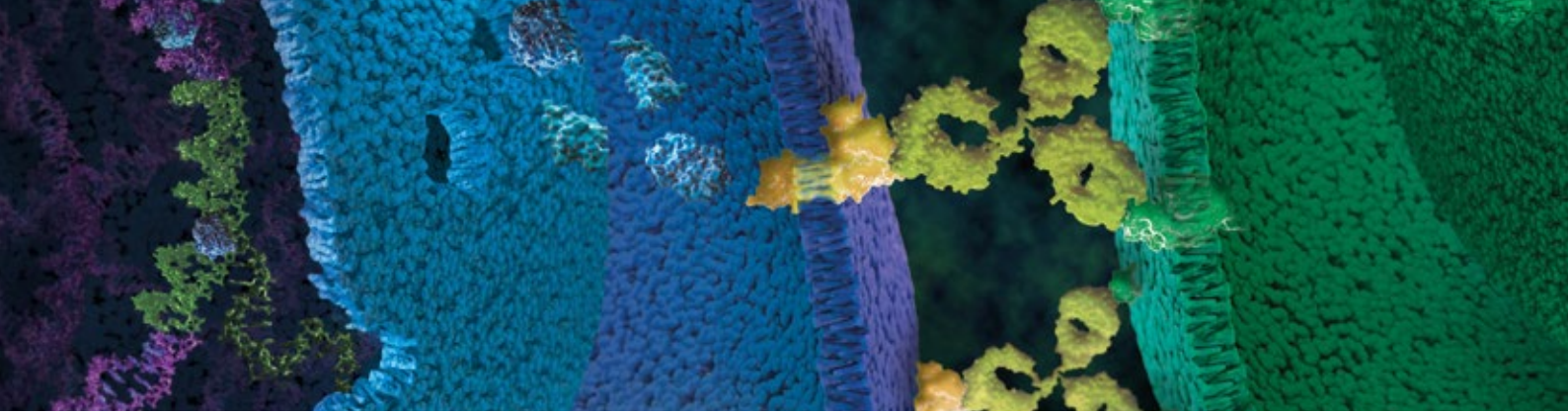
The bioassay is prequalified according to ICH guidelines and shows the precision, accuracy and linearity required for routine use in potency and stability studies. In addition, the bioassay workflow is simple and robust and compatible with both 96-well and 384-well plate formats used for antibody screening 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 PD-1/PD-L1 Blockade Bioassay reflects the MOA of biologics designed to block the PD-1/PD-L1 interaction

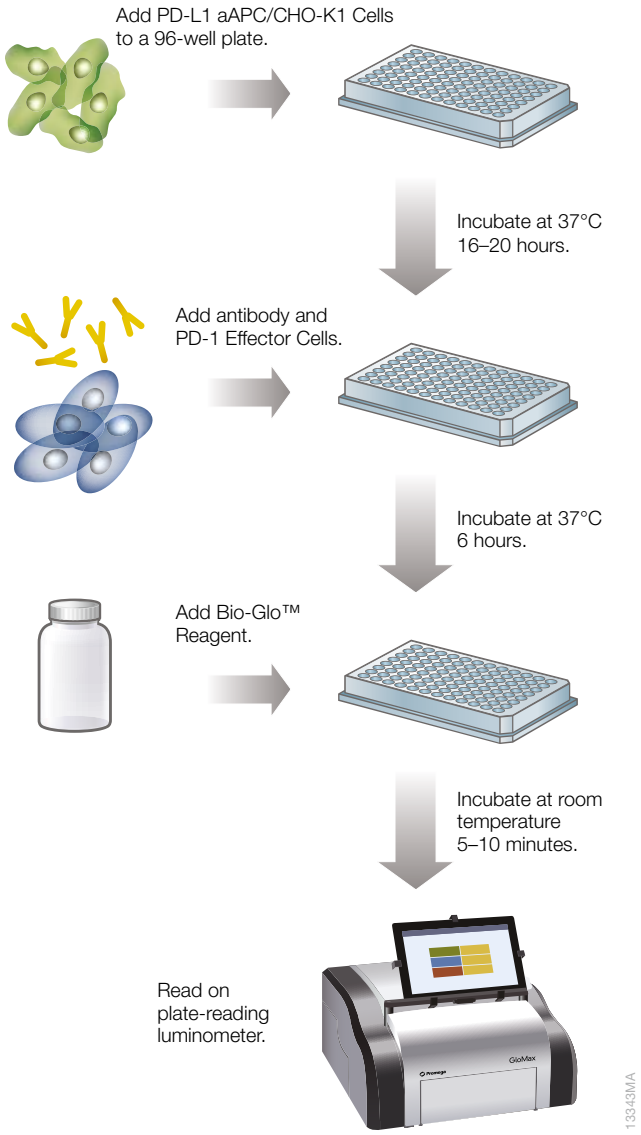


The bioassay consists of two genetically engineered cell lines, PD-1 Effector Cells and PD-L1 aAPC/CHO-K1 Cells. When co-cultured, the TCR is activated but the PD-1/PD-L1 interaction inhibits TCR-mediated luminescence. When the PD-1/PD-L1 interaction is disrupted, regained TCR activation induces luminescence (via activation of the NFAT pathway) that can be detected by addition of Bio-Glo™ Reagent and quantitation with the GloMax® Detection System.

* artificial Antigen Presenting Cell (aAPC)

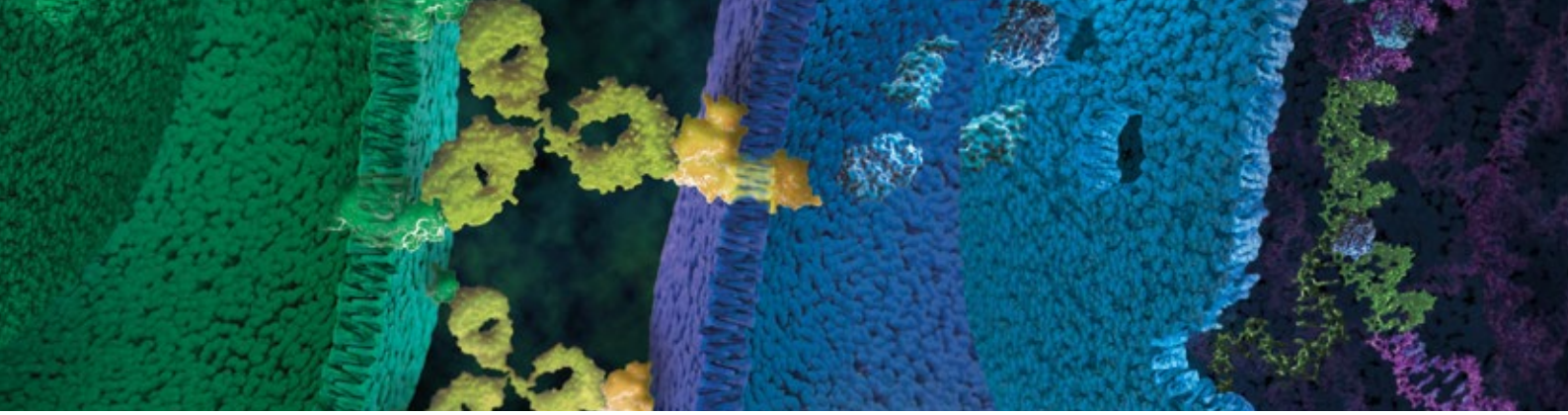


Schematic protocol for the PD-1/PD-L1 Blockade Bioassay

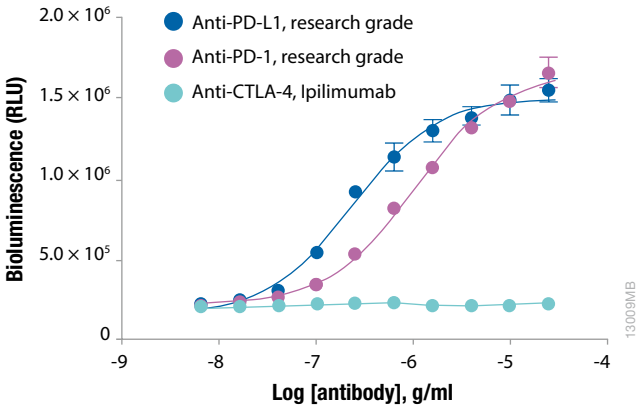


Convinient "Thaw-and-use" cell format, no cell culture required

The PD-1/PD-L1 Blockade Bioassay combines (1) a simple, add-mix-read single-day workflow with PD-1 Effector Cells and PD-L1 aAPC/CHO-K1 Cells provided in a frozen, "thaw-and-use" format and an optimized protocol, that together yield a quantitative bioassay that exhibits low variability and high accuracy.

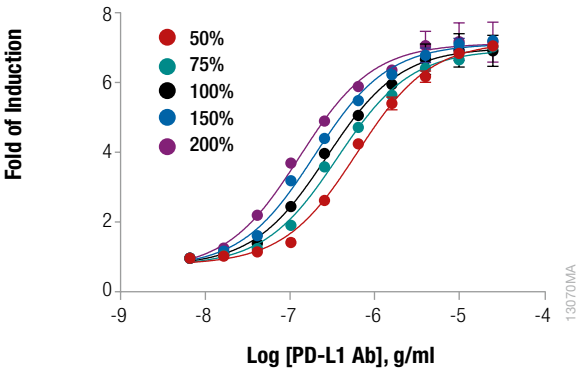


The PD-1/PD-L1 Blockade Bioassay is specific for PD-1 and PD-L1 antibodies

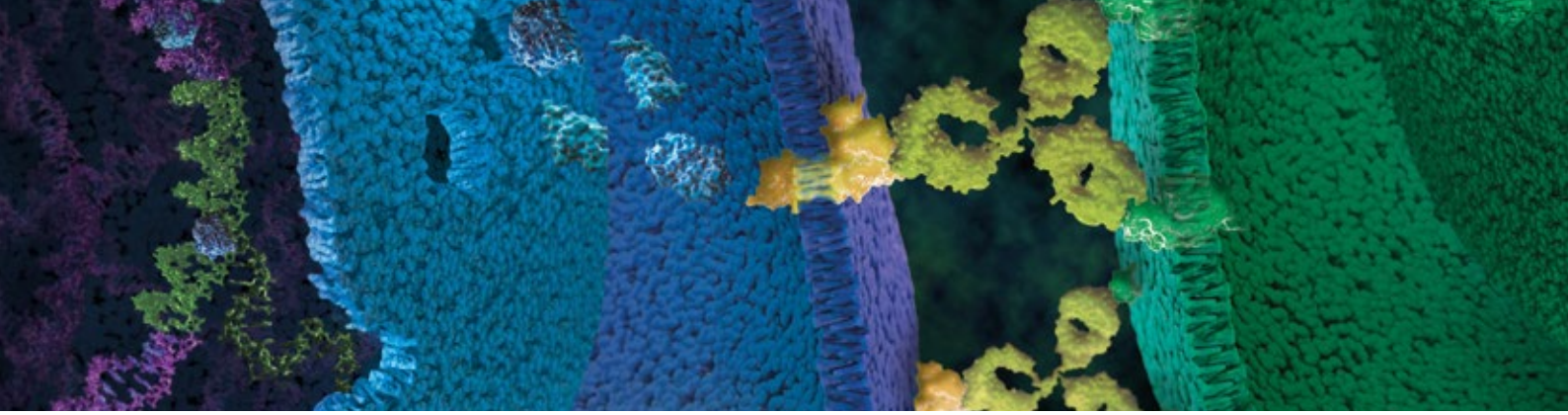


PD-1 Effector Cells and PD-L1 aAPC Cells were incubated for 6 hrs at 37°C with increasing concentrations of either an anti-PD-1, PD-L1 or CTLA-4 antibody. The anti-PD-1 and PD-L1 antibodies, but not the anti-CTLA-4 antibody, blocked the immune checkpoint inhibitory signal resulting in luciferase activity.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

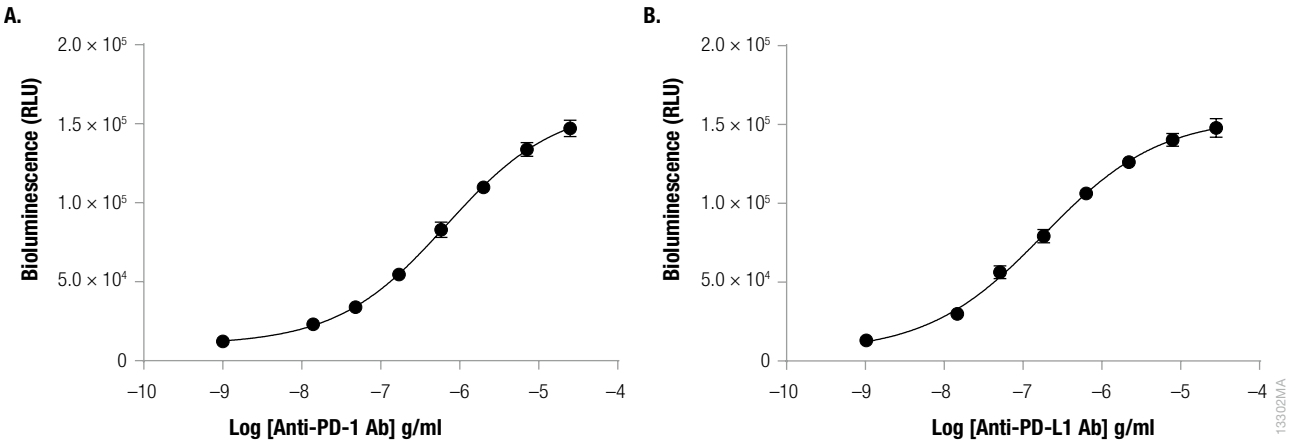
The PD-1/PD-L1 Blockade Bioassay is accurate and reproducible over a relative potency range of 50–200 %



PD-1 Effector Cells and PD-L1 aAPC Cells were incubated with increasing concentrations 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.



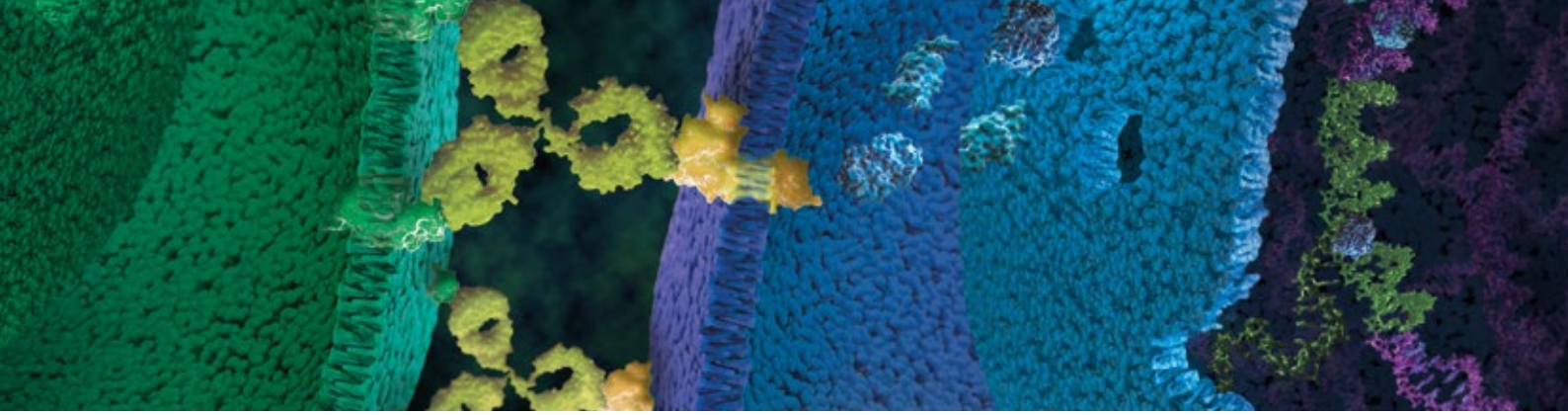
The assay is amenable to 384-well plate format and compatible with automation



Control Ab, 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. Bio-Glo™ Reagent was added and luminescence quantified using the GloMax® Detection System. Data were fitted to a 4PL curve using GraphPad Prism® Software. Data were generated using "thaw-and-use" cells.

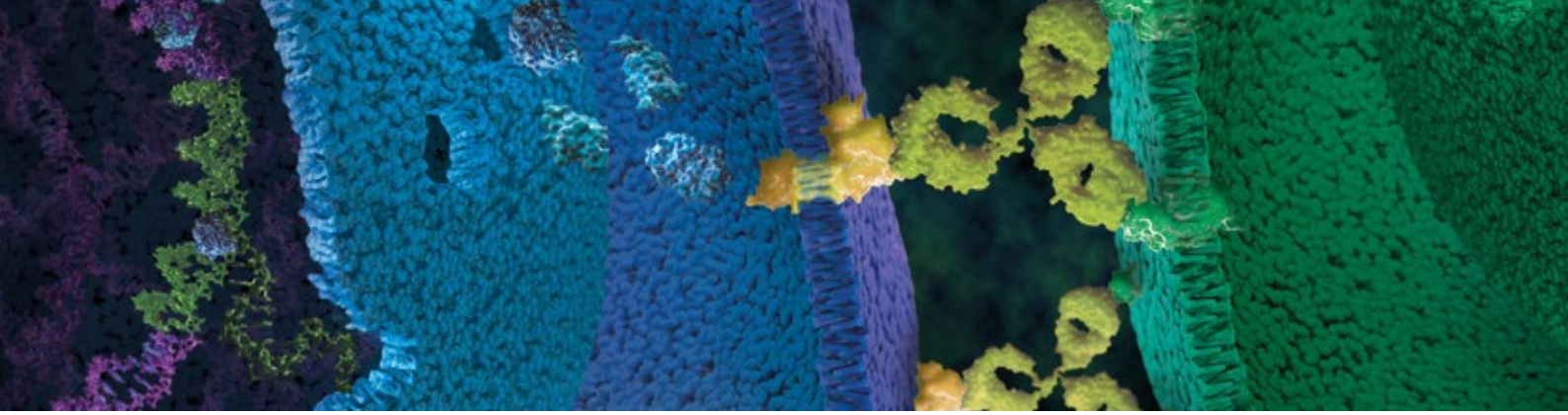
Immune Checkpoint Bioassays for other Targets

As mentioned above, Promega developed a wide panel of immune checkpoint assays other than PD1/PDL1. These include co-inhibitory (e.g. PD-1/PD-L2, CTLA-4, TIGIT, LAG-3) and co-stimulatory (e.g. GITR, 4-1BB (CD137), OX40, CD40) receptors as well as potential combinatory assays. The assay principles reflect the MOA of the appropriate targets by measuring the recovery of a signal by disrupting inhibition for co-inhibitory targets or measuring an activation for the co-stimulatory targets. All these assays are available in "thaw-and-use" format and thereby give all the benefits in terms of low variability, easy handling, good precision, accuracy and linearity required for potency and stability measurements. 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.

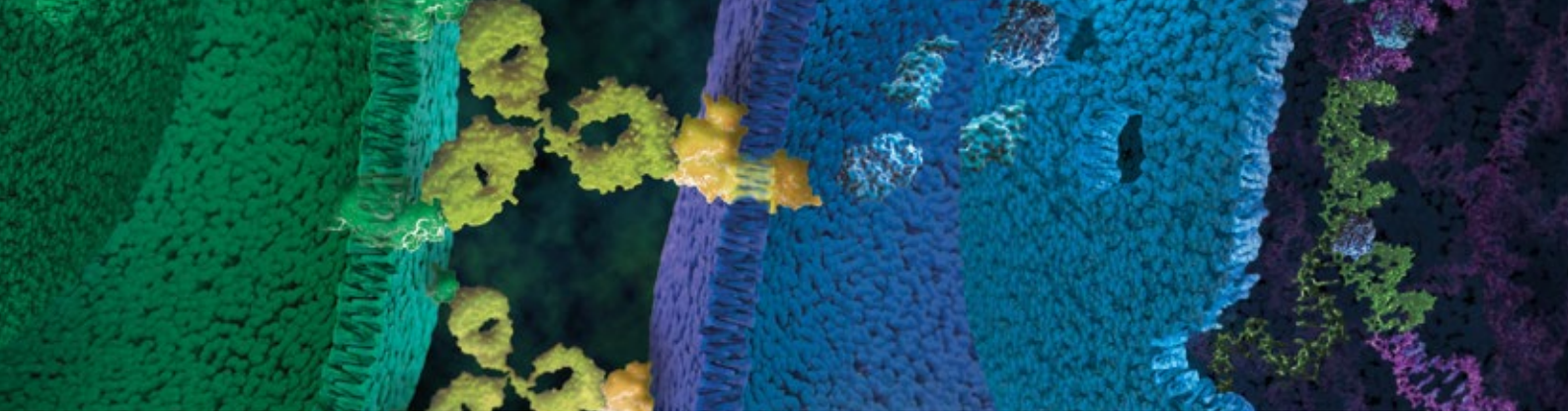


Immune Checkpoint Bioassay: Ordering information

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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
PD-1/PD-L1 Blockade Bioassay, Propagation Model	Catalog	J1252	2 x 1 vial PD-1 Effector Cells (CPM) 2 x 1 vial PD-L1 aAPC/CHO-K1 Cells (CPM)	No limit
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 Cell	No limit
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 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate 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 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate 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	No limit



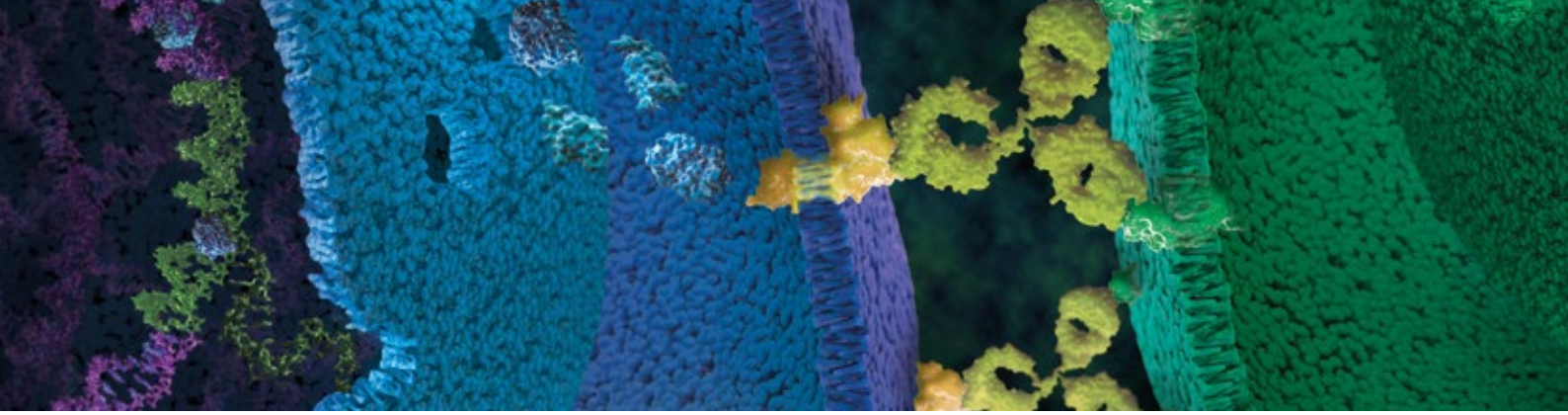
CTLA-4 Blockade Bioassay	Product Category	Cat. No.	Components	Assays in 96-well format
CTLA-4 Blockade Bioassay Kit	CAS	CS186920	CTLA-4 Effector Cells and aAPC/Raji Cells 1 x 1 vial CTLA-4 Effector Cells 1 x 1 vial aAPC/Raji Cells 4 ml Fetal Bovine Serum 1 x 36ml RPMI 1640 Medium 1 x 10ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
CTLA-4 Blockade Bioassay Kit 5X	CAS	CS186926	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 10ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
CTLA-4 Blockade Bioassay, Propagation Model	CAS	CS186906	CTLA-4 Effector Cells aAPC/Raji Cells	No limit
LAG-3 Blockade Bioassay				
LAG-3 Blockade Bioassay Kit *This version of the LAG-3 Blockade Bioassay includes LAG-3 Effector Cells (Jurkat) and "primed" Raji Cells. "Primed" refers to the pre-incubation of the Raji cells with superantigen (SEE) prior to cryopreservation.*	CAS	CS194802	1 x 1 vial LAG-3 Effector Cells 1 x 1 vial Primed Raji Cells 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
LAG-3 Blockade Bioassay Kit 5X	CAS	CS194806	5 x 1 vial LAG-3 Effector Cells 5 x 1 vial Primed Raji Cells 5 x 4 ml Fetal Bovine Serum 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo Luciferase Assay System 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
LAG-3 Blockade Bioassay, Propagation Model *This version of the bioassay requires an own superantigen (SEE or SED)*	CAS	CS194801	2 x 1 vial LAG-3 Effector Cells Target cells are Raji (not provided)	No limit
TIGIT Blockade Bioassay				
TIGIT/CD155 Blockade Bioassay Kit	CAS	CS198807	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 Ham's F12 Medium 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
TIGIT/CD155 Blockade bioassay Kit 5X	CAS	CS198811	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 Ham's F12 Medium 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo Luciferase Assay System 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
TIGIT/CD155 Blockade Bioassay, Propagation Model	CAS	CS198801	2 x 1 vial TIGIT Effector Cells 2 x 1 vial CD155 aAPC/CHO-K1 Cells	No limit



Co-Stimulatory Bioassays	Product Category	Cat. No.	Components	Assays in 96-well format
GITR Bioassay				
GITR Bioassay Kit *New and improved version that utilizes luc2 (vs. luc2P)*	CAS	CS184006	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
GITR Bioassay Kit 5X	CAS	CS184009	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
GITR Bioassay, Propagation Model	CAS	CS184004	2 x 1 vial GITR Effector Cells	No limit

4-1BB (CD137) Bioassay				
4-1BB Bioassay Kit *New and improved version that utilizes luc2 (vs. luc2P)*	CAS	CS196005	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
4-1BB Bioassay Kit 5X	CAS	CS196008	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrat	600
4-1BB Bioassay, Propagation Model	CAS	CS196004	2 x 1 vial 4-1BB Effector Cells	No limit

OX40 Bioassay				
OX40 Bioassay Kit *New and improved version that utilizes luc2 (vs. luc2P); replaces CS197701*	CAS	CS197704	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
OX40 Bioassay Kit 5X	CAS	CS197707	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
OX40 Bioassay, Propagation Model	CAS	CS197703	2 x 1 vial OX40 Effector Cells	No limit



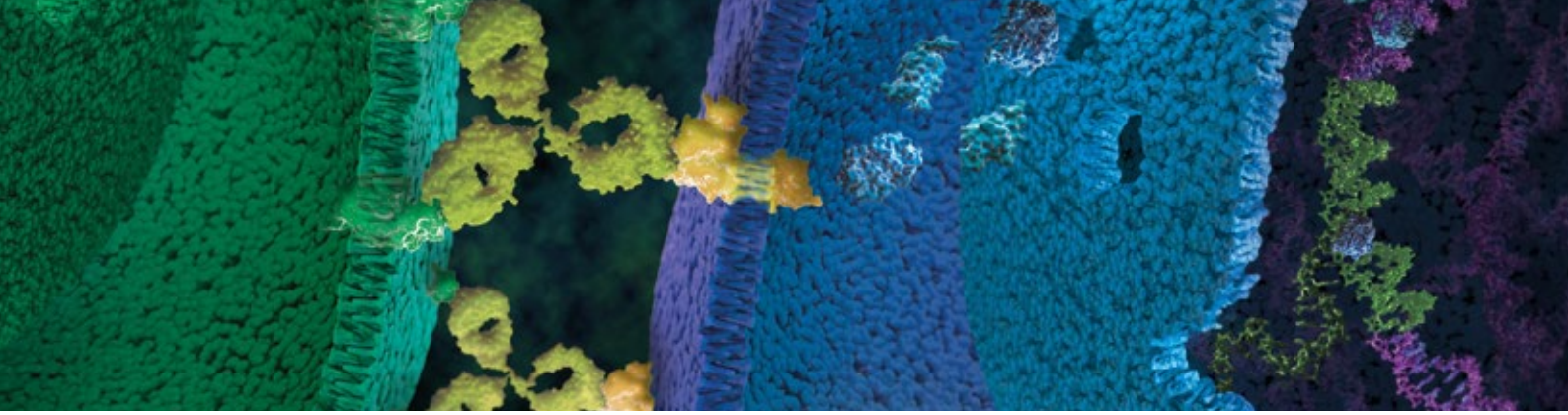
CD40 Bioassay	Product Category	Cat. No.	Components	Assays in 96-well format
CD40 Bioassay Kit *New and improved version that utilizes luc2 (vs. luc2P) and U2OS cells (vs. Ramos cells)*	CAS	CS1979A06	1 x 1 vial CD40 Effector Cells (U2OS) 1 x 4 ml Fetal Bovine Serum 1 x 36 ml RPMI 1640 Medium 1 x Bio-Glo Luciferase Assay System 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
CD40 Bioassay Kit 5X	CAS	CS1979A12	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 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
CD40 Bioassay, Propagation Model	CAS	CS1979A03	2 x 1 vial CD40 Bioassay Cells (U2OS)	No limit

FcγRIIb/CHO-K1 Cell Line *(optional cell line for use with co-stimulatory bioassays)*				
FcγRIIb/CHO-K1 Cell Line, Propagation Model	CAS	CS1979A05	2 x 1 vial FcgRIIb/CHO-K1 Cells	No limit

HVEM/LIGHT Bioassay				
HVEM/LIGHT Bioassay, Propagation Model	CAS	CS1979A10	2 x 1 vial HVEM Bioassay Cells	No limit

CD27 Bioassay				
CD27 Bioassay, Propagation Model	CAS	CS1979A15	2 x 1 vial CD27 Bioassay Cells	No limit

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



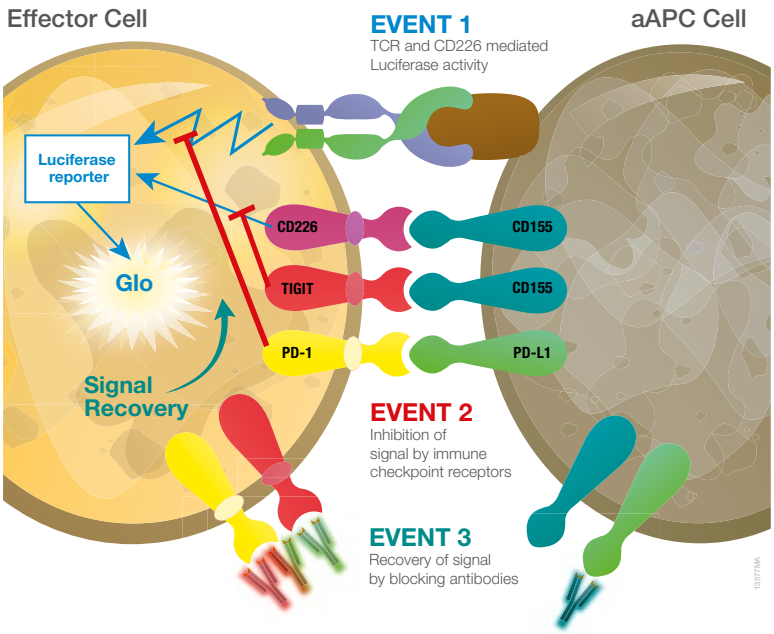
Bioassays Power Combination Immunotherapy

Therapeutic antibodies designed to block immune checkpoint receptors or activate immunostimulatory receptors are a promising strategy to treat cancer and function by modulating a patient's own immune system. Co-engagement of multiple immune receptors on chronically activated T cells, known as combination immunotherapy, can potentially elicit better therapeutic outcomes through a combinatorial effect compared with engagement of a single receptor.

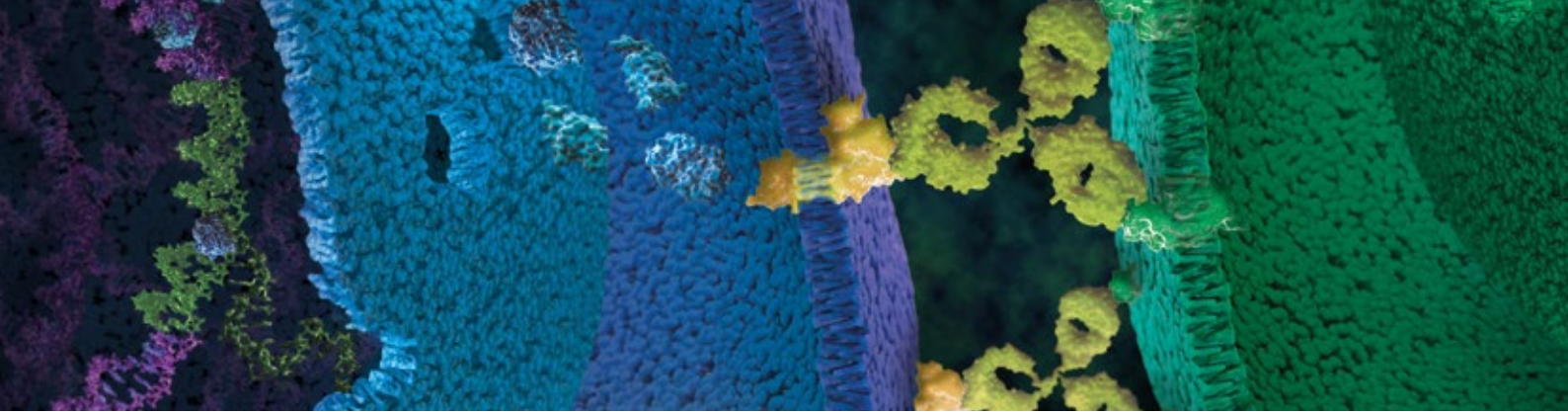
PD-1 and TIGIT immune checkpoint antibody development

The results of numerous clinical trials support the strategy of combination immunotherapy. For example, PD-1/PD-L1 blocking antibodies which have shown positive clinical results, are now being evaluated in combination with antibody drugs that target additional immune checkpoint receptors. TIGIT is a target immune checkpoint receptor that is expressed on subsets of activated T cells and natural killer (NK) cells. The ligand for TIGIT is CD155 (also called poliovirus receptor) that is, interestingly, also a ligand for CD226, an immune co-stimulatory receptor involved in antiviral and antitumor responses. TIGIT negatively modulates NK cell killing and T cell activation via two mechanisms: competing with CD226 for binding to CD155 on adjacent cells and directly preventing CD226 homodimerization and signaling.

Schematic representation of the blockade bioassays for PD-1/PD-L1, TIGIT/CD155 and PD-1 + TIGIT

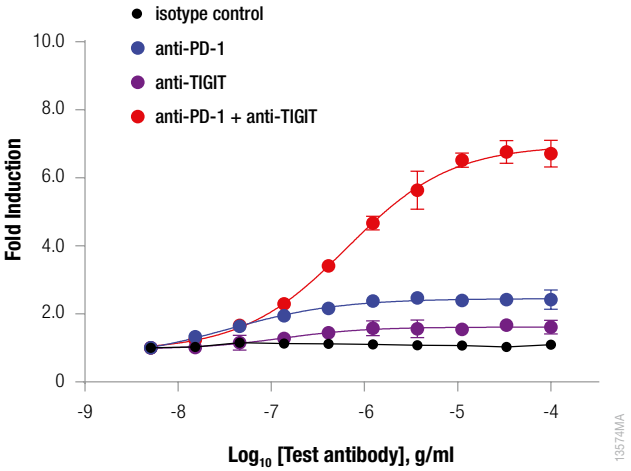


Each bioassay consists of a T Effector Cell (expressing PD-1, TIGIT or PD-1 + TIGIT) and an aAPC cell (expressing PD-L1, CD155 or PD-L1 + CD155), respectively. Co-culture of a T Effector Cell with its corresponding aAPC cell leads to three events as noted in the cell image.



Synergy of the PD-1+TIGIT Combination Bioassay

The PD-1+TIGIT Combination Bioassay uses the same assay principle as the blockade bioassays mentioned above. Here, the T Effector Cells co-express PD-1 and TIGIT (PD-1+TIGIT Effector Cells) and aAPC cells co-express PD-L1 and CD155 (PD-L1+CD155 aAPC Cells).



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.

Immune Checkpoint Bioassay: Ordering information

PD-1+TIGIT Combination Bioassay	Product Category	Cat. No.	Components	Assays in 96-well format
PD-1+TIGIT Combination Bioassay Kit	CAS	CS198813	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 1 x 10 ml Bio-Glo Luciferase Assay Buffer 1 x 1 vial Bio-Glo Luciferase Assay Substrate	120
PD-1+TIGIT Combination Bioassay Kit 5X	CAS	CS198817	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 5 x Ham's F12 Medium 5 x 36 ml RPMI 1640 Medium 5 x Bio-Glo Luciferase Assay System 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600
PD-1+TIGIT Combination Bioassay, Propagation Mode	CAS	CS198802	2 x 1 vial PD-1+TIGIT Effector Cells 2 x 1 vial PD-L1+CD155 aAPC/CHO-K1 Cells	No limit

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

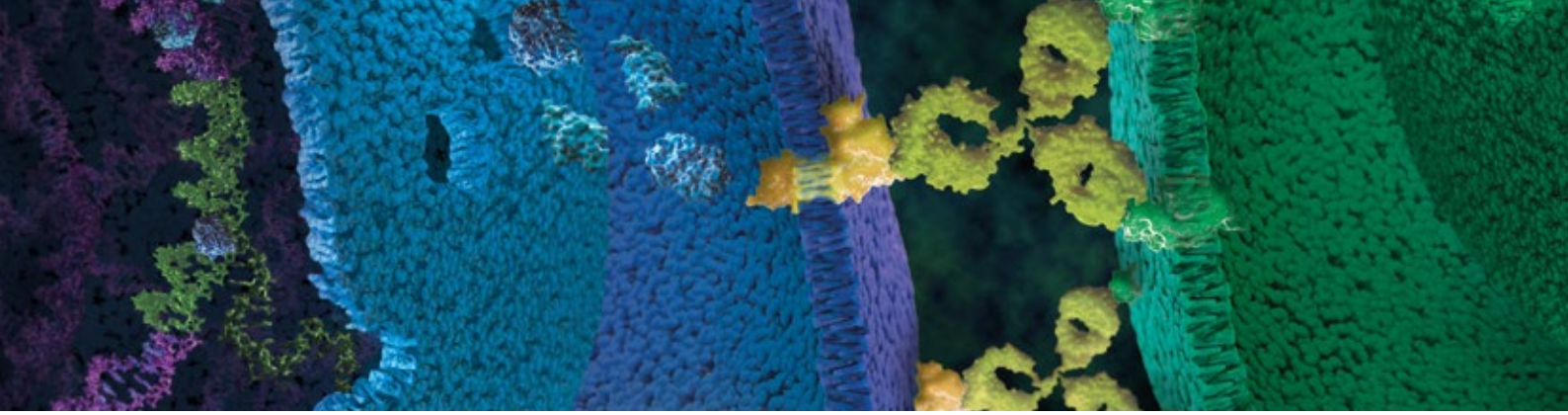
Growth Factor & Cytokine Bioassays

TNFα Blocker Bioassay Suitability for Validation for QC Lot-Release and Stability

TNFα blocker biopharmaceuticals (e.g. Etanercept, Infliximab) represent an important and successful class of protein drugs used in the treatment of several autoimmune diseases. Promega has developed a low variability, homogeneous and robust bioluminescent TNFα blocker drug bioassay based on quantification of caspase-3 activity. It addresses the mechanism of action of blocking TNFα-mediated effects on the induction of caspase-mediated apoptosis. Bioassay cells were developed into a convenient single-use, frozen "thaw-and-use" format and in doing so potential variability arising from pre-assay culture was eliminated. The bioassay is suitable for potency determination and stability testing. It also meets the ICH Guidelines Q2 (R1) requirements for precision, linearity and range, repeatability, accuracy and specificity.

VEGF/KDR Cell-Based Bioassay for Quantifying and Monitoring Activity of Members of the VEGF Family of Ligands and Antibody-mediated Blockade of VEGF Binding to KDR

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulate vasculogenesis and angiogenesis. VEGF-mediated signalling occurs in tumour cells and this signalling contributes to key aspects of tumorigenesis such as cancer stem cells function and tumour initiation. The role of VEGF in cancer is mainly but not limited to angiogenesis and vascular permeability. Effects of VEGF are mediated by VEGF receptors like the kinase insert domain receptor (KDR) also known as vascular endothelial growth factor receptor 2 (VEGFR-2). Promega VEGF/KDR cell-based bioassay is a luciferase reporter assay that can be conveniently employed for quantifying and monitoring activity of members of the VEGF family and also antibody-mediated blockade of VEGF binding to KDR. The Promega engineered HEK293 reporter cell-based VEGF/KDR bioassay replaces the cumbersome primary HuVEC cell-based assays.



Growth Factor & Cytokine Bioassays: Ordering information

Growth Factor & Cytokine 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 Blockade Apoptosis Bioassay Designed for use with Promega's Caspase-Glo3/7 Assay Reagents (Cat#G8091)	CAS	CS1324A05	TNFα Receptor Cells (U937)	120
Membrane TNFα Bioassay Cross-listed in the Fc Effector tab under anti-TNFα ADCC Bioassay	CAS	CS185502	Membrane TNFα Cells (CHO-K1)	120
Membrane TNFα Bioassay, Propagation Model Cross-listed in the Fc Effector tab under anti-TNFα ADCC Bioassay	CAS	CS185501	Membrane TNFα Cells (CHO-K1)	No limit

KDR/VEGF Bioassay				
KDR/VEGF Bioassay Kit	CAS	CS181403	1 vial KDR/VEGF HEK293 cells 4 ml Fetal Bovine Serum – 4mL 36 ml DMEM 1x 10 ml Bio-Glo™ Luciferase Assay Buffer 1 vial Bio-Glo Luciferase Assay Substrate	120
KDR/VEGF Bioassay Kit, 5X	CAS	CS181407	5 vials KDR/VEGF HEK293 cells, 5 x 4 ml Fetal Bovine Serum 5 x 36 ml DMEM 5 x 10 ml Bio-Glo Luciferase Assay Buffer 5 x 1 vial Bio-Glo Luciferase Assay Substrate	600

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

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.de/products/fluorometers-luminometers-multimode-readers

Streamline Your Antibody Enrichment Using Scalable Magnetic Bead-Based Chemistries

Manual or Automated Antibody Purification from Different Sample Types

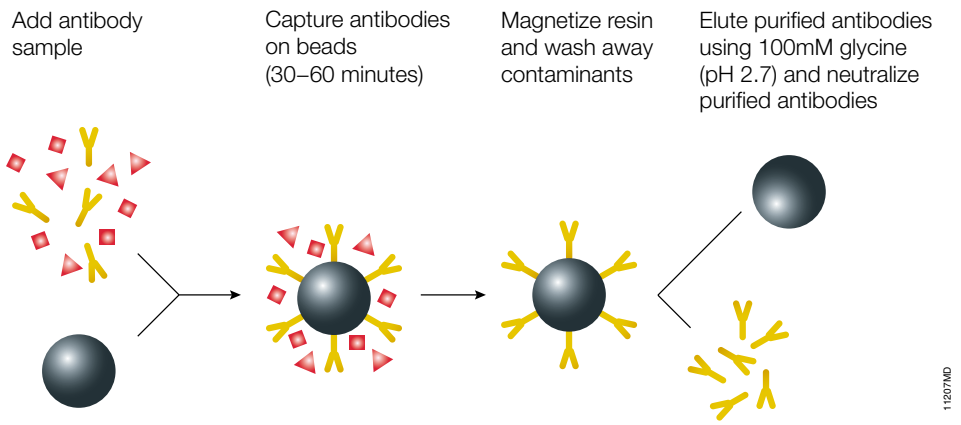
Magne™ Protein A and Magne™ Protein G Beads are magnetic affinity beads with high specificity and improved capacity for binding antibodies from cell culture supernatant, ascites fluid and serum samples. Paramagnetic beads are composed of iron encapsulated in macroporous cellulose with low non-specific 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.

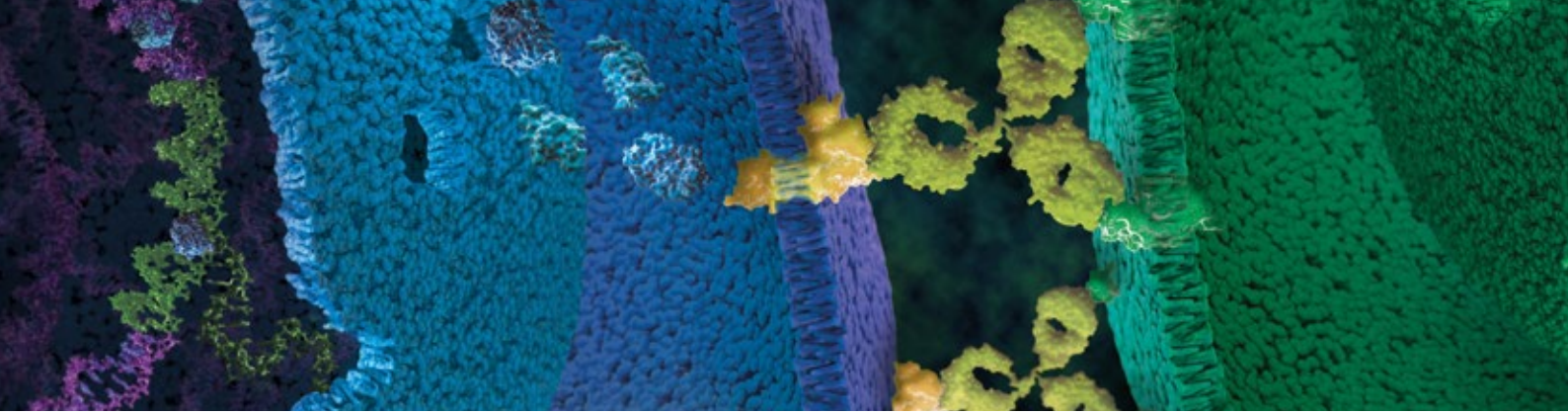
Antibody purification and concentration from small-volume or dilute source material

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.

Automated, high-throughput antibody purification

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.

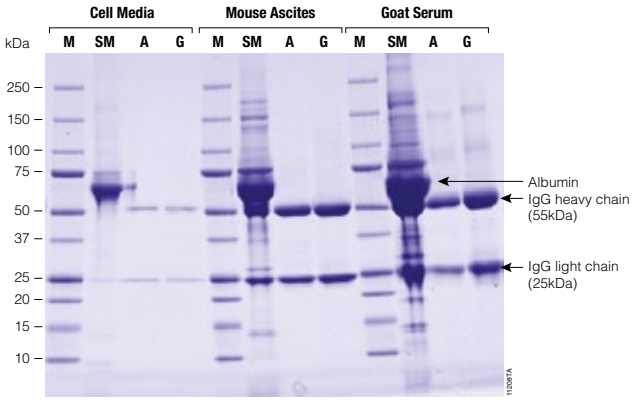
Optimized performance

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) using published protocols (TM371). Samples were separated via SDS-PAGE by adding 1µl of starting material (SM) or 5µl of purified sample (A or G) and stained with Coomassie®-based stain.

Ordering information

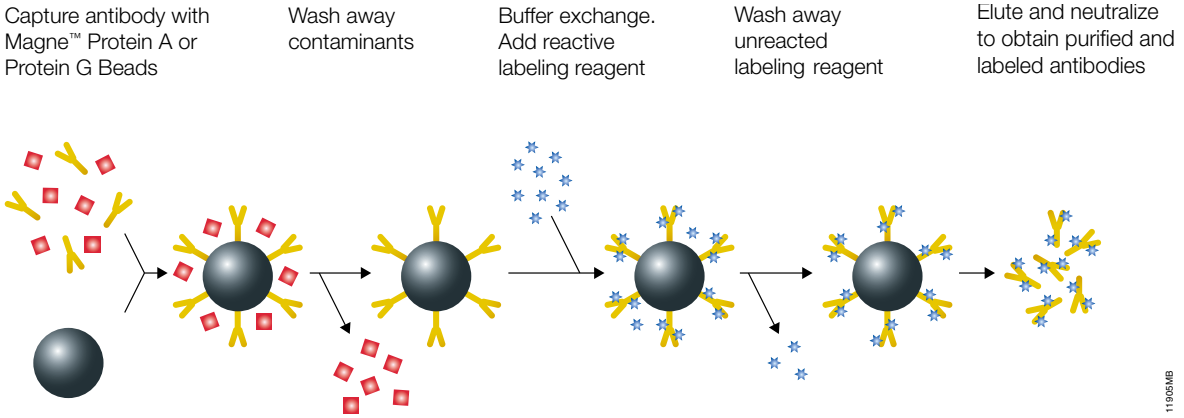
Product	Size	Cat. No.
Magne™ Protein G Beads, 20% Slurry	1 ml	G7471
Magne™ Protein G Beads, 20% Slurry	5 ml (5 × 1 ml)	G7472
Magne™ Protein G Beads, 20% Slurry	50 ml	G7473
Magne™ Protein A Beads, 20% Slurry	1 ml	G8781
Magne™ Protein A Beads, 20% Slurry	5 ml (5 × 1 ml)	G8782
Magne™ Protein A Beads, 20%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

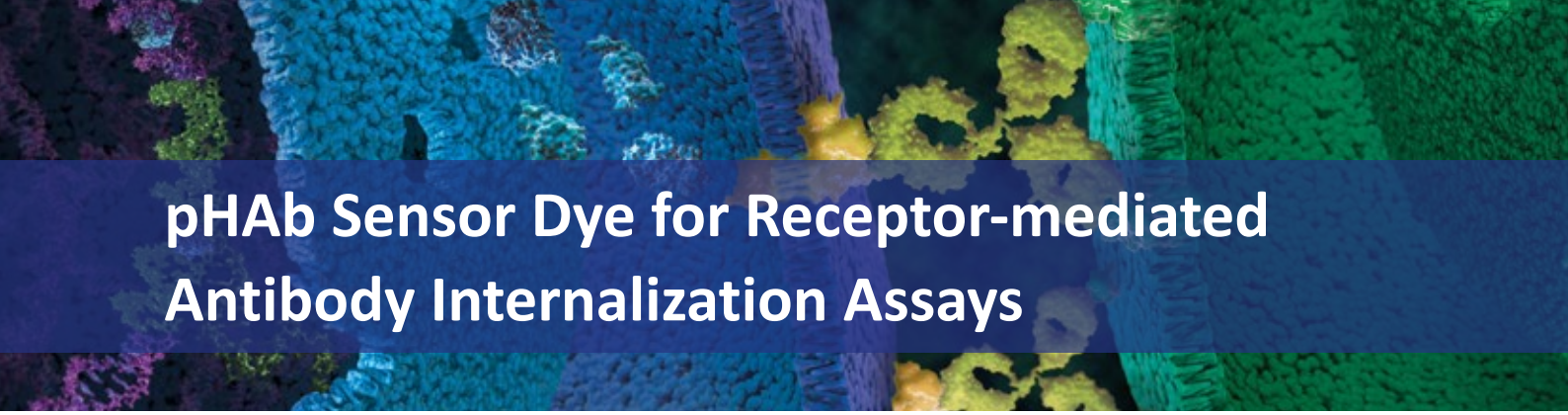
Anibody conjugation is required for many different applications including the generation of Antibody Drug Conjugates (ADCs). To simplify the workflow for antibody conjugation and to increase throughput, high capacity Magne™ Protein A and Magne™ Protein G beads can be used for on-bead antibody conjugation. 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) assay.

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 for Receptor-mediated Antibody Internalization Assays

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.

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. Internalization of antibodies can be recorded with a plate-reader, FACS and (confocal) microscopy.

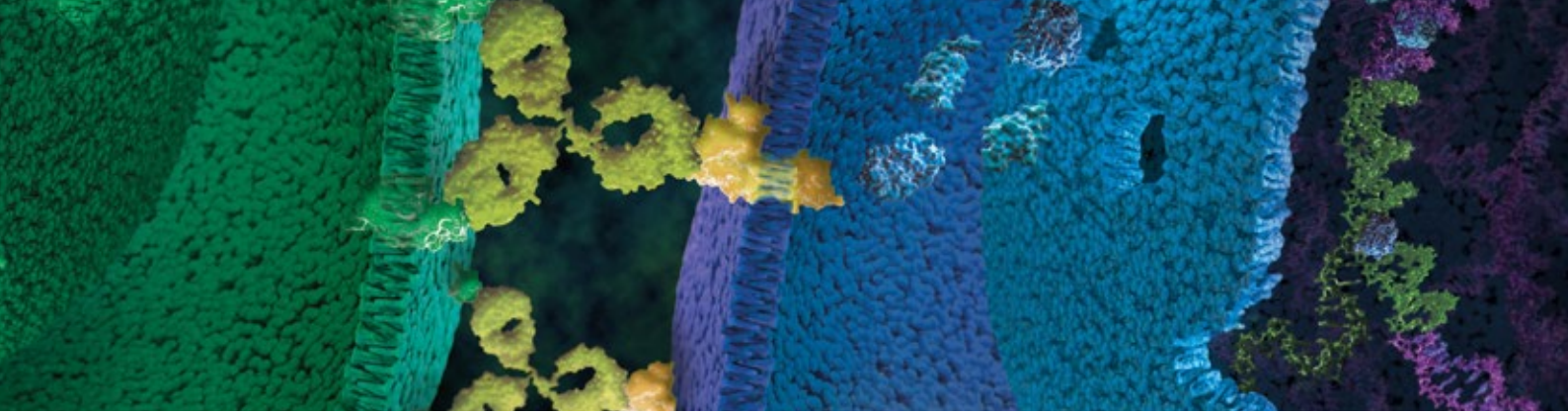
Features & benefits

- 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 × 250 µg	G9841
pHAb Amine Reactive Dye	4 × 250 µg	G9845
pHAb Thiol Reactive Dye	1 × 250 µg	G9831
pHAb Thiol Reactive Dye	4 × 250 µg	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) L to R
Cat. No. Z5331, Z5332, Z5333

Twelve-position. Up to two sample volumes (50 µl – 1.0 ml) L to R
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

Simplified 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

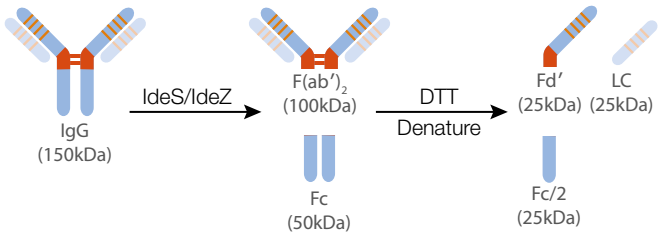
Fast and easy: Digestion in 30 minutes with no optimization

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

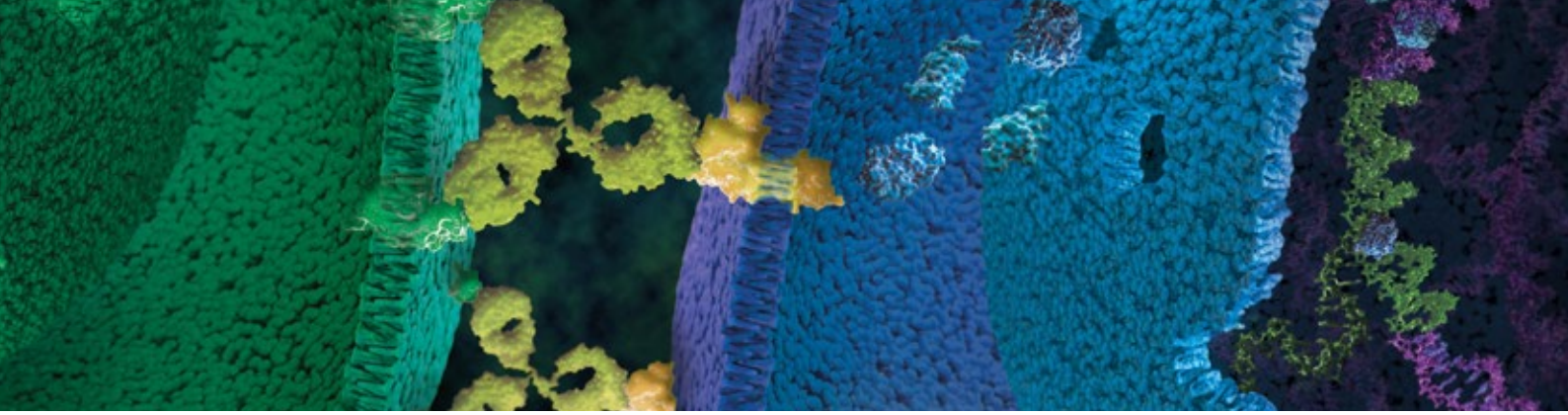


Ordering information

Product	Size	Conc.	Cat. No.
Ides Protease	5.000 units	lyophilized	V7511
Ides Protease	25.000 units (5 x 5000 units)	lyophilized	V7515
Ides Protease, Frozen	2.000 units	50 u/µl	V7512
IdeZ Protease	5.000 units	lyophilized	V8341
IdeZ Protease	25.000 units (5 x 5000 units)	lyophilized	V8345
Ides Protease, Frozen	1 mg	50 u/µl	V8342

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, repectively.

For Research Use Only. Not for Use in Diagnostic Procedures



Suppression of Sample Preparation-induced Artefacts

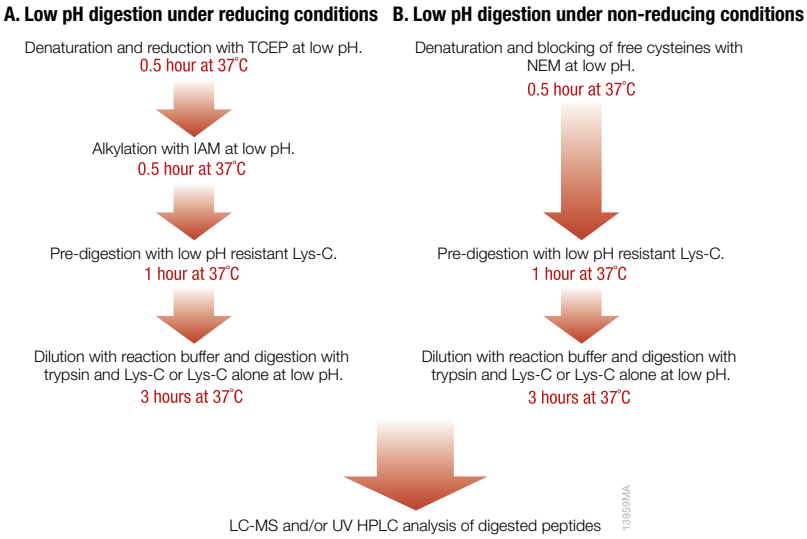
AccuMAP™ Low pH Protein Digestion Kit

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.

Biologics

Bioassays for biologics

Functional assays for potency evaluation and manufacturing lot release are essential tools in the development of therapeutic antibody biologics. Promega's Reporter Bioassay platform allows simple and robust evaluation of an antibody's ability to activate the Fc effector function mechanism which triggers ADCC or ADCP. Based on a simple microplate-based reporter assay format, the Promega Fc-Receptor Reporter Bioassays use convenient "thaw-and-use" cells to simplify assay planning.

To enable characterisation of therapeutic antibodies, Promega's Custom Assay Services team have also developed a suite of pathway specific cell-based bioassays for a range of targets including immune checkpoint blockade targets such as CTLA-4 and PD1/PD-L1 etc.

Purification tools

Promega's paramagnetic beads for antibody purification use a novel attachment chemistry to immobilise recombinant Protein G or Protein A molecules in an oriented fashion on the surface of a bead with low non-specific binding properties. With a range of magnetic separation solutions available, Magne™ Protein G and Magne™ Protein A beads can be used as the basis of a manual or automated approach for achieving high purity and high recovery of monoclonal and polyclonal antibodies from a variety of biological samples including cell culture media, ascites and serum. Easily scalable (20 µl – 50 ml), magnetic beads also allow "on bead" conjugation of bound antibodies with other molecules eg. toxic payloads for ADCs and Promega's superior pH sensor dyes for tracking antibody/ADC internalisation by fluorescence microscopy.

Protein characterisation tools

Biologics require extensive analytical characterisation at the molecular level due to the potential for post-translational modification and chemical changes that can occur during storage and handling. Promega has a comprehensive portfolio of tools for the analysis of biologics using mass spectrometry including proteases and glycosidases.

Tools for antibody drug conjugates

ADCs need to be effectively internalised into tumour cells in order to release their toxic payload. Promega's pH sensor dyes fluoresce as surface membrane-bound antibody is internalised into low pH endosomal compartments. Coupled with Promega's RealTime-Glo™ Metabolic Cell Viability Assay technology you can study the kinetics of both ADC internalisation and the onset of cellular toxicity.

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