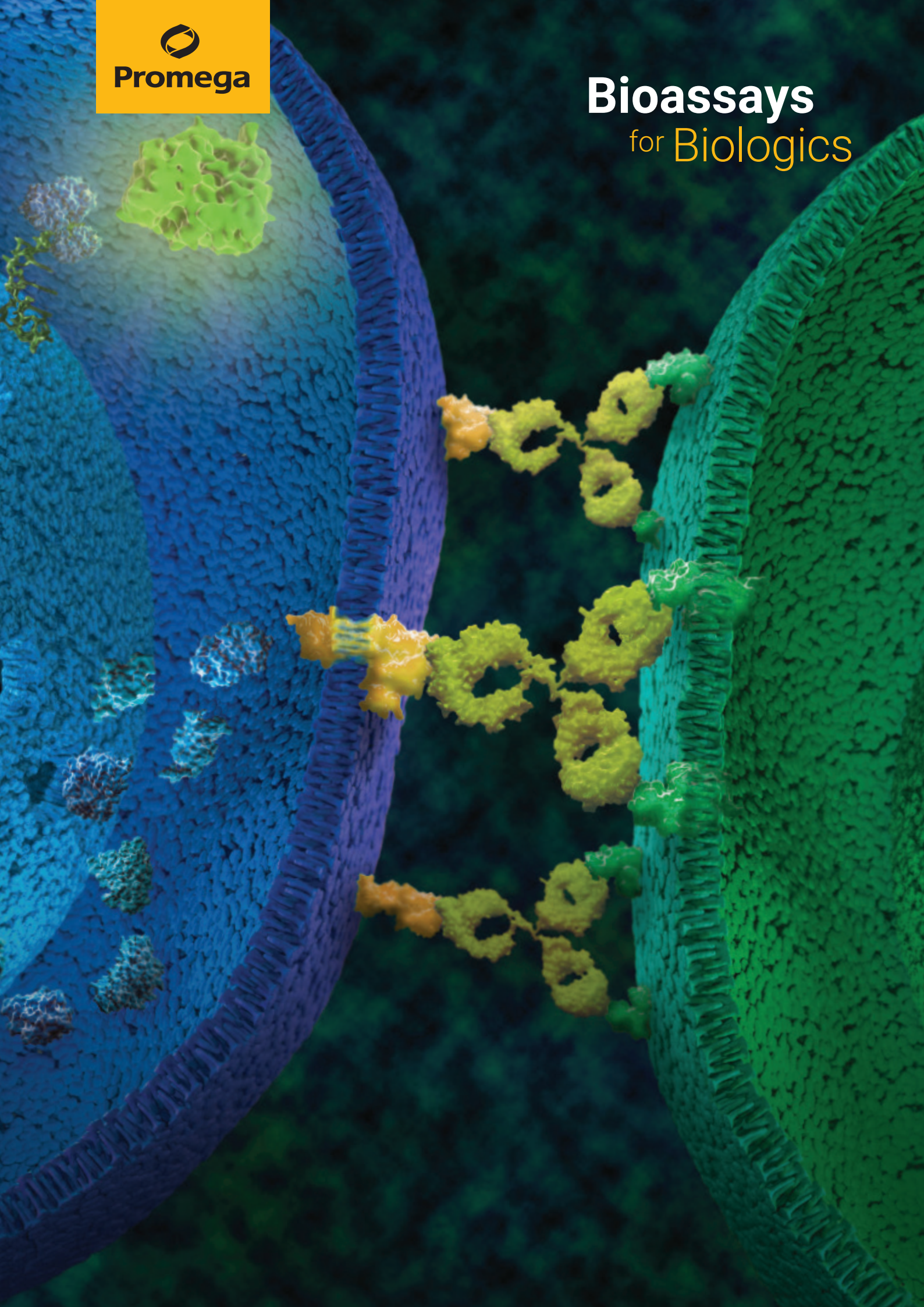
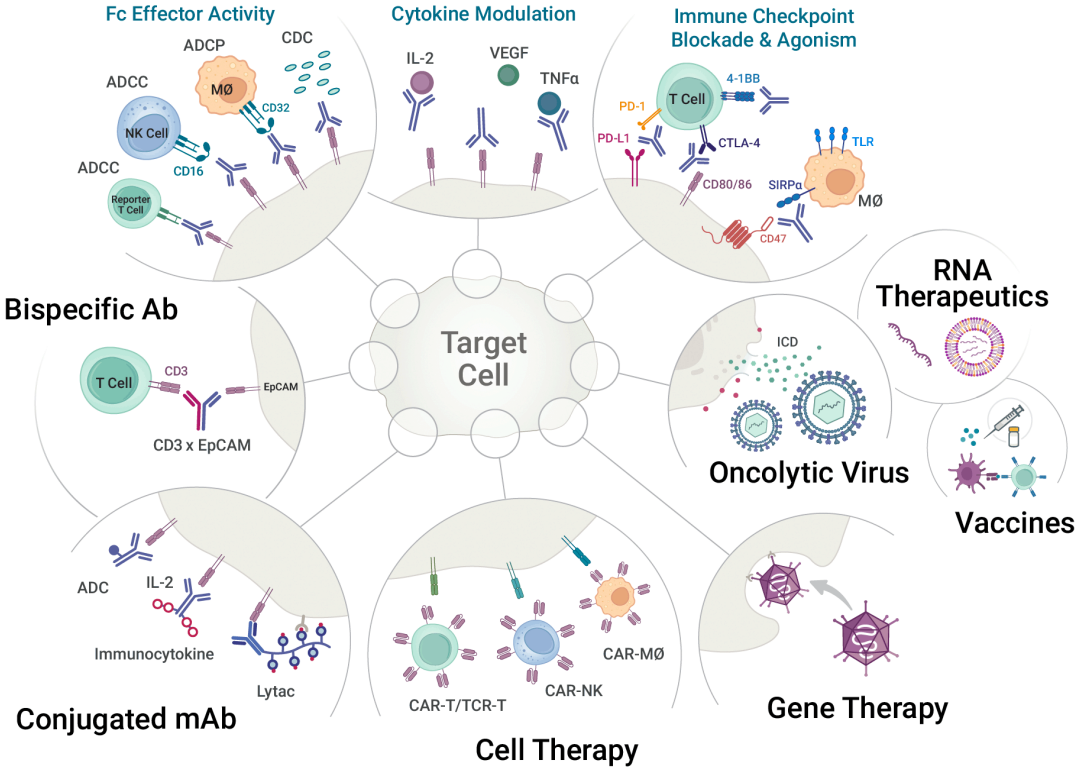
Promega

# Bioassays for Biologics



## A Multipronged Approach to Biologic Therapeutics

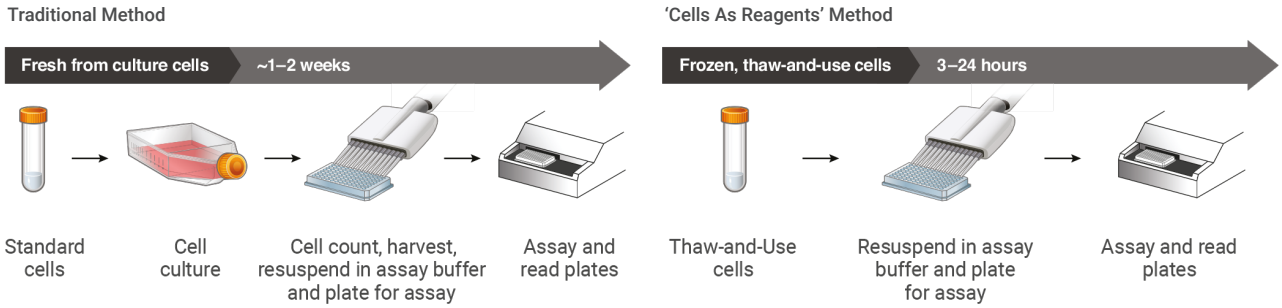
Biologic drug developers employ a rapidly growing range of approaches to the treatment of disease. Understanding a drug's mechanism of action (MOA) is key to successful development. The use of MOA-based bioassays to measure the potency and stability of drug candidates is critical throughout the drug development workflow.




In contrast to small-molecule drugs that are chemically synthesised and have a known structure, biologics are drug products with complex, heterogeneous structures that are often unstable and sensitive to external conditions. Due to their high degree of complexity, the development of biologics requires a comprehensive set of quantitative, accurate and precise bioanalytical tools.

Promega offers an extensive toolbox of reporter bioassays to characterise and develop novel monoclonal antibody (mAb)-based therapeutics, cell and gene therapies, vaccines and RNA therapeutics. These assays are useful for interrogating a range of biological functions, including Fc effector activity, immune checkpoint modulation, T-cell activation, cytokine and growth factor signalling and innate immune regulation. 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.

## Promega Bioassays Offer a Rapid and Simple Workflow Compared to Traditional Methods




## 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.

## Promega Bioassays Follow a Simple, 'Add-mix-read' Format.


Plate bioassay cells → Add test Ab → 6- to 24-hour induction → Add Bio-Glo™ Reagent → Measure luminescence (e.g., using GloMax® Discover Instrument)

# Fc Effector Bioassays

## Benefits

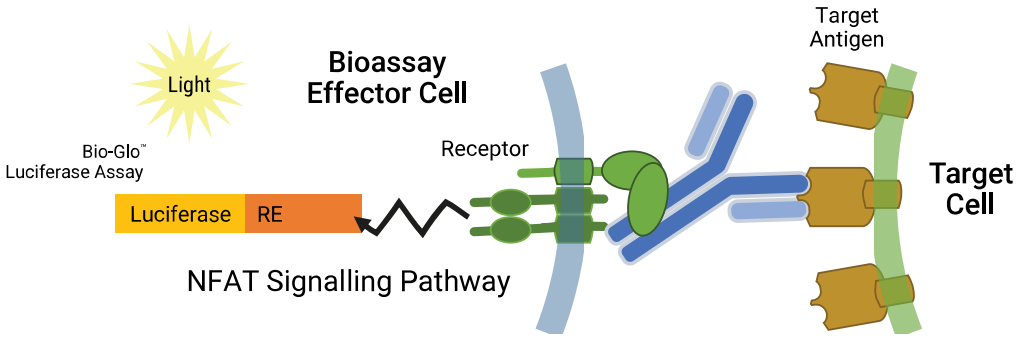
- Cell-based reporter bioassay platform to measure ADCC and ADCP mediated through Fcγ receptors
- Cells express specific Fcγ receptors and NFAT response element, which drive luciferase-based reporters
- No reliance on inconsistent primary peripheral blood mononuclear cells (PBMCs)
- Scalable measurement 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

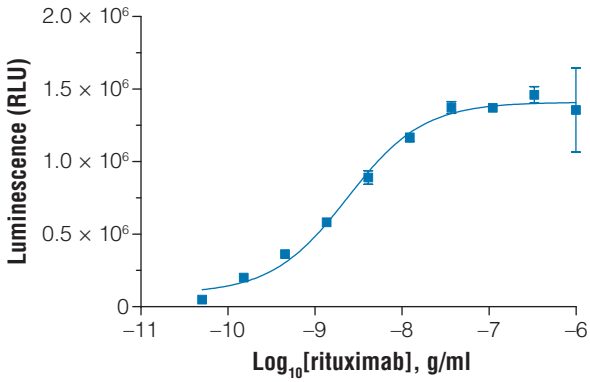
Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important mechanism of action (MOA) of antibodies that target virus-infected or diseased (e.g., tumour) cells for destruction by components of the cell-mediated immune system. Fc receptor-mediated effects contribute to the efficacy and safety of therapeutic antibodies to tumour 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 response element.

The bioassays overcome the limitations of more labour-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.




**ADCC Reporter Bioassay response to rituximab (RITUXAN®) using ADCC Bioassay Effector Cells.** CD20+ WIL2-S cells were harvested and plated in a 96-well assay plate followed by addition of a series of concentrations of rituximab. ADCC Bioassay Effector Cells were then added to the assay plate. After 20 hours of induction at 37°C, Bio-Glo™ Luciferase Assay Reagent was added and luminescence was determined.

## 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**, 121905.

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.

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.

# Immune Checkpoint Modulation Bioassays

## Benefits

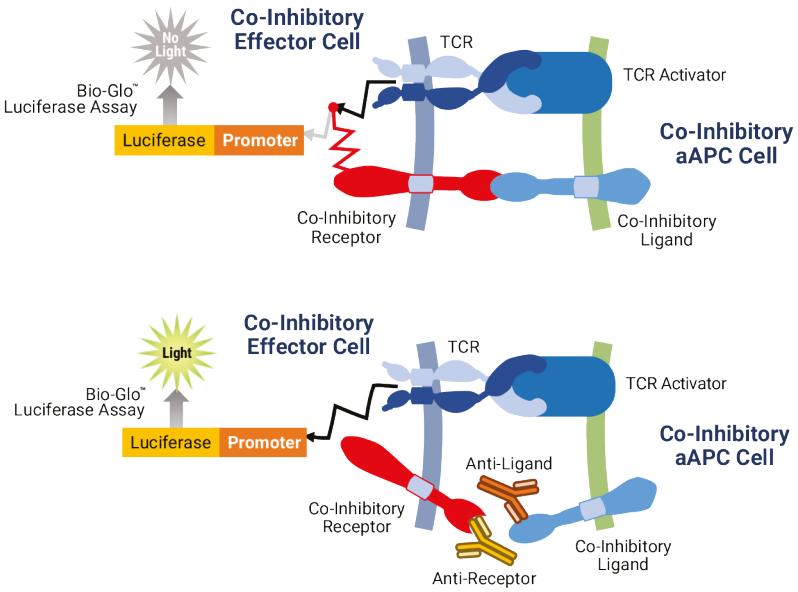
- Provide quantitative measures of immune checkpoint blockade or agonist activity
- Combination bioassays support development of therapeutic approaches targeting more than one immune checkpoint receptor.

## Overview

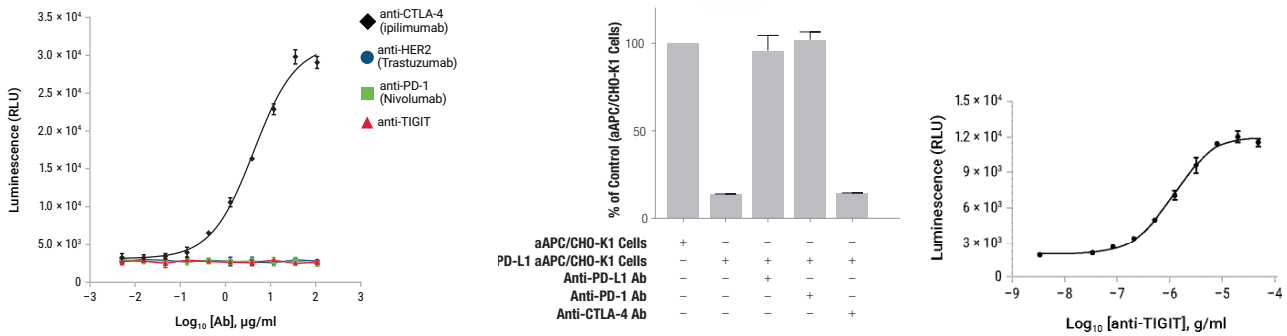
### Co-Inhibitory Receptor Bioassays

Immune inhibitory receptors expressed on activated T cells and B cells play a critical role in regulating immune responses to tumour antigens and autoantigens. The human immune system is comprised of a complex network of such co-inhibitory and co-stimulatory pathways. Engagement of a receptor by its ligand on an adjacent cell inhibits T-cell receptor (TCR) signalling 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.

These biologically relevant, MOA-based assays can be used to measure the potency and stability of antibodies and other biologics designed to block the interaction between immune inhibitory receptors and their ligands.



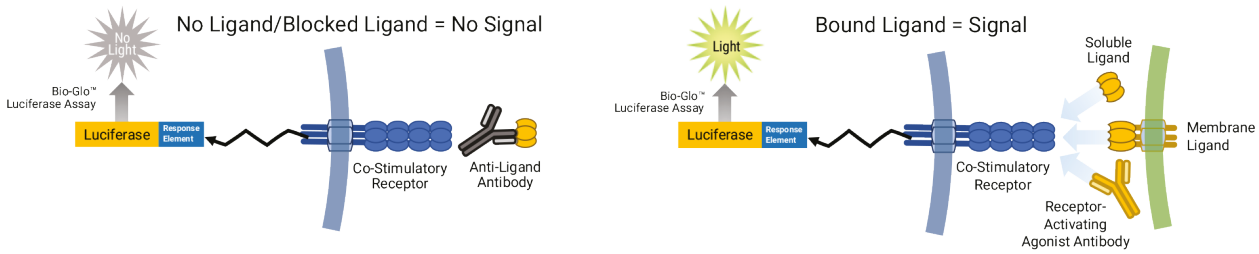

**Co-inhibitory bioassay principle.** The bioassay typically consists of two genetically engineered cell lines: 1) an effector cell that expresses the receptor; and 2) an artificial antigen-presenting cell (aAPC) that expresses the ligand. During co-culture, the TCR is activated by the TCR activator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



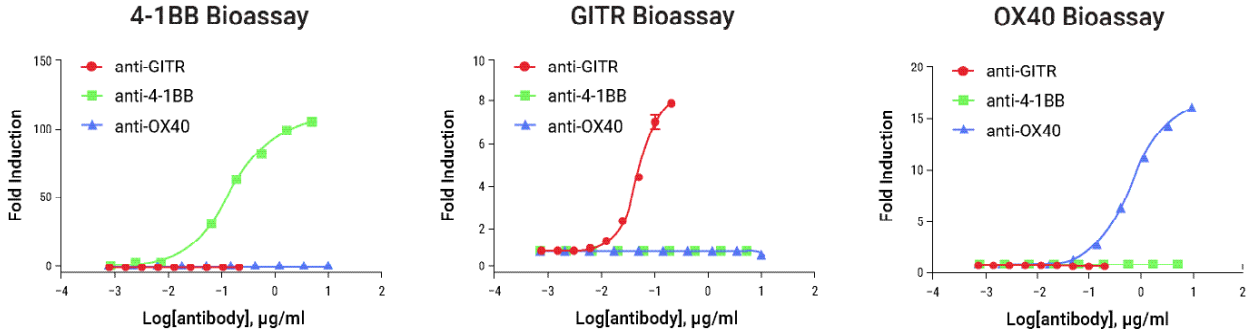

**Antibody potency determination.** Bioassays for CTLA-4 (left), PD-1/PD-L1 (centre) and TIGIT/CD155 (right) blockade showed high sensitivity and specificity in measuring the potency of monoclonal antibodies against these co-inhibitory targets.

## 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.




**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.




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

# T Cell Activation Bioassays

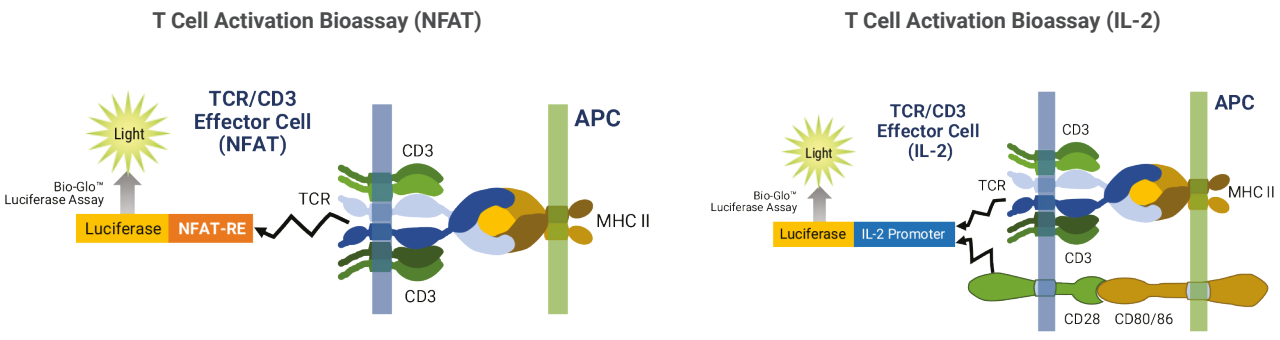
## Benefits

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

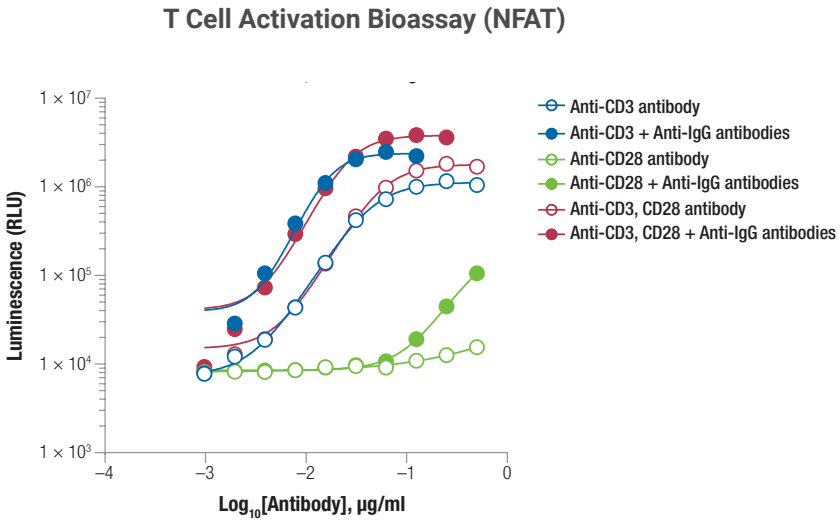
## Overview

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.

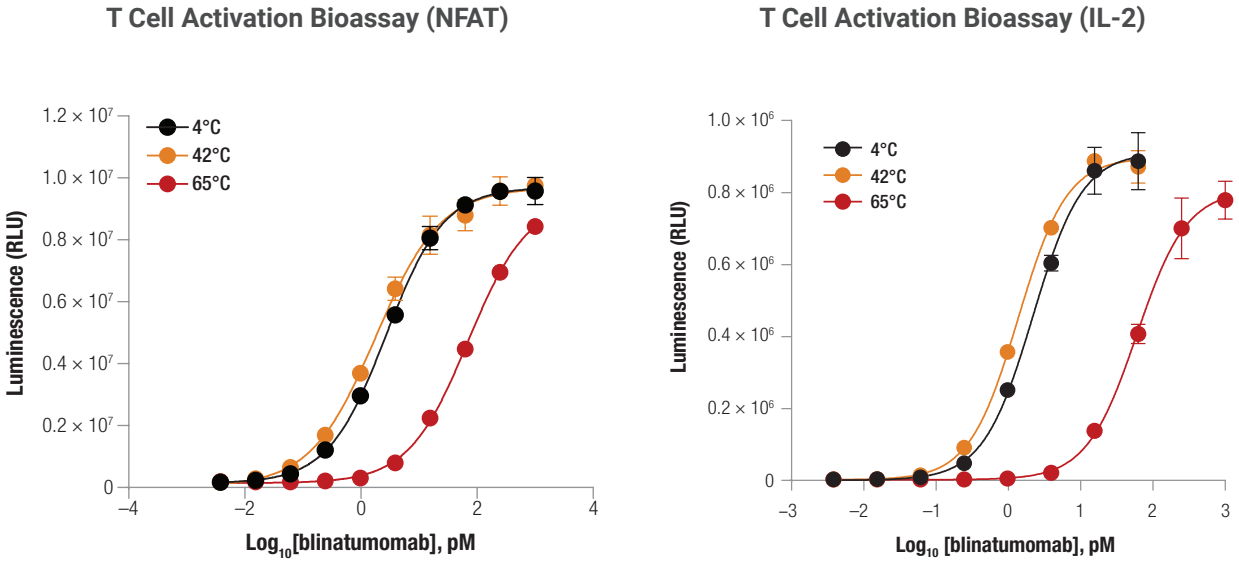
The T Cell Activation Bioassays are bioluminescent cell-based assays that overcome limitations of inconsistencies posed by existing assays. They can be used for the discovery and development of anti-CD3 bispecific molecules and to evaluate the activity of exogenously expressed chimeric antigen receptor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




**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 signalling results in IL-2 promoter-mediated luminescence.




**The T Cell Activation Bioassay reflects the mechanism of action (MOA) of biologics designed to engage the TCR and induce TCR-mediated T cell activation.** TCR/CD3 Effector Cells (NFAT) 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.




**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 analysed using the T Cell Activation Bioassay (NFAT or IL-2, as indicated).

# Cytokine and Growth Factor Bioassays

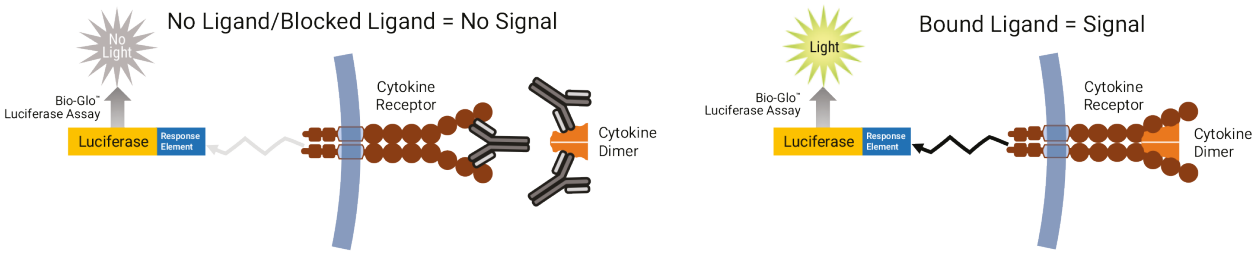
## Benefits

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

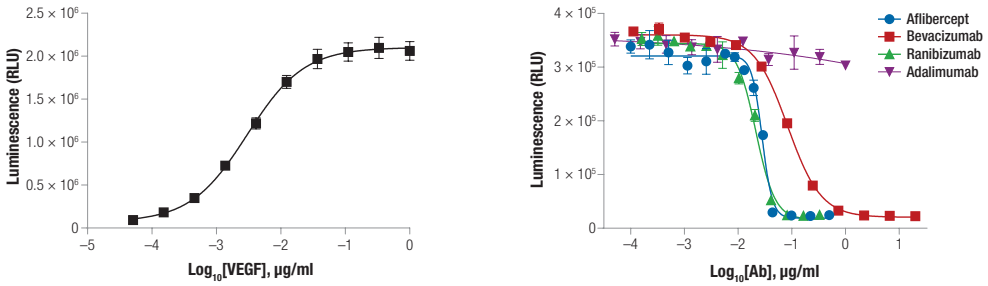
## Overview

Complex networks of cytokines and growth factors are involved in multiple cellular signalling 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, TGFβ, IFNβ and IFNγ and several interleukins, including IL-2, IL-6, IL-12, IL-15, IL-17 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).

# Biologics Assay Development and Services

## Develop Custom Bioassays for Your Target of Interest

### New Assay Development

- Potency assays for mAb therapeutics, CAR-T cell therapy, gene therapy
- Genetic reporter bioassays, PPI bioassays for novel targets
- Target cells for target cell killing assays (ADCC, CAR-T cells)
- Immunoassays (e.g., AAV)
- PsVLP assays (e.g., RSV).

### Modifications of Existing Assays

- KO or ectopic expression of targets and/or pathway molecules
- Expression of customer-specific TCRs in TCR-KO cell lines
- Non-human versions of existing assays (cow, pig, dog).

### Drug Profiling/Characterisation

- Testing customer biologics (mAb, CAR-T cells) in existing assays
- PBMC ADCC Bioassays (for bridging studies to reporter bioassays).

### Bioassay Qualification

- Assay optimisation and bioassay qualification of customer drug
- Thaw-and-Use cell lot qualification
- Support to transfer assays/reagents to customer or CRO for Validation.

### Custom Cell Manufacture

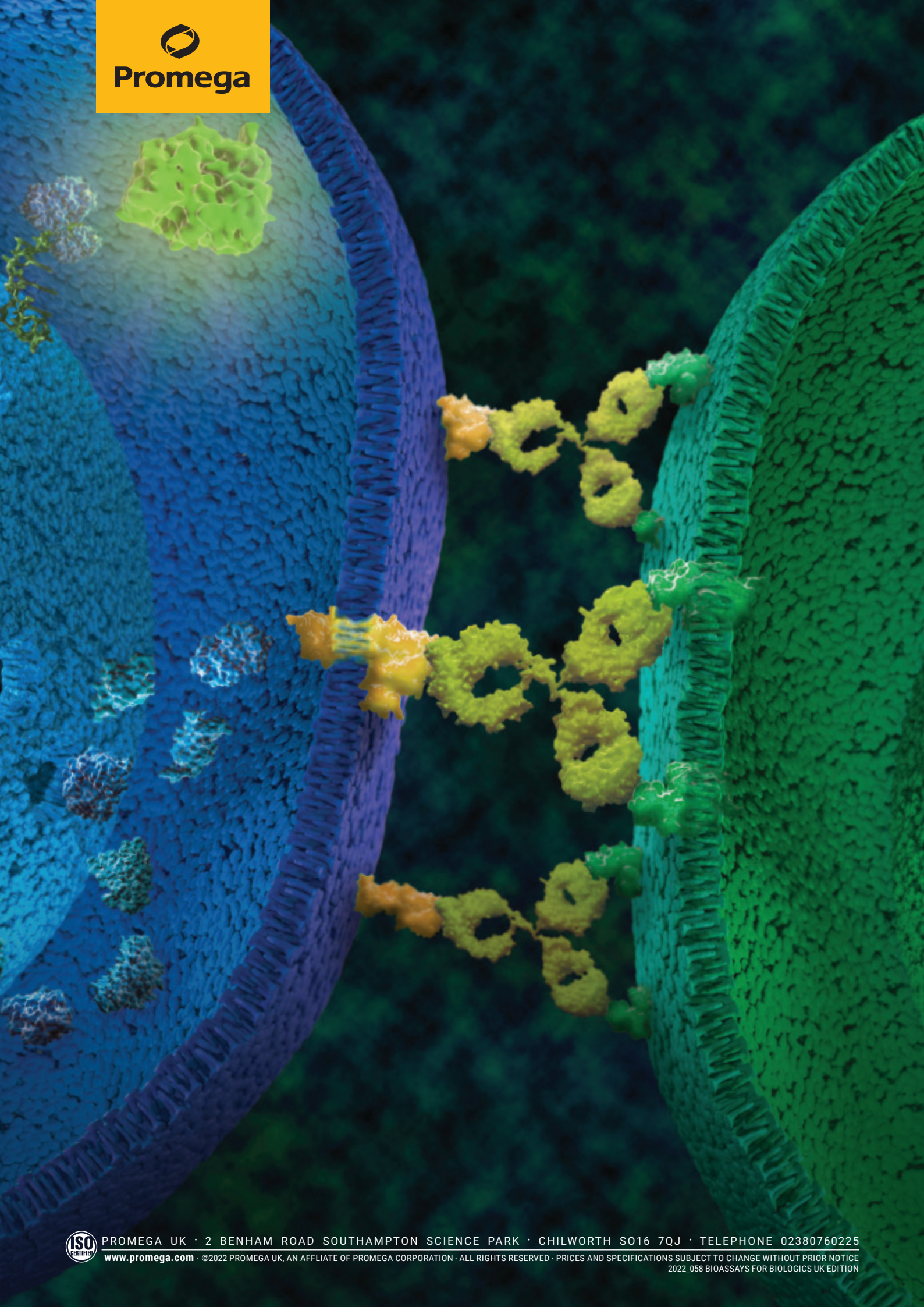
- Master Cell Banks
- Custom dispense of bioassay Thaw-and-Use cells.

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 enquiries, e-mail:
**TailoredSolutions@promega.com**

For more information, visit:
**www.promega.com/custom-bio**

To learn more about Promega bioassays for biologics, visit:
**www.promega.com/BetterBioassay**



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