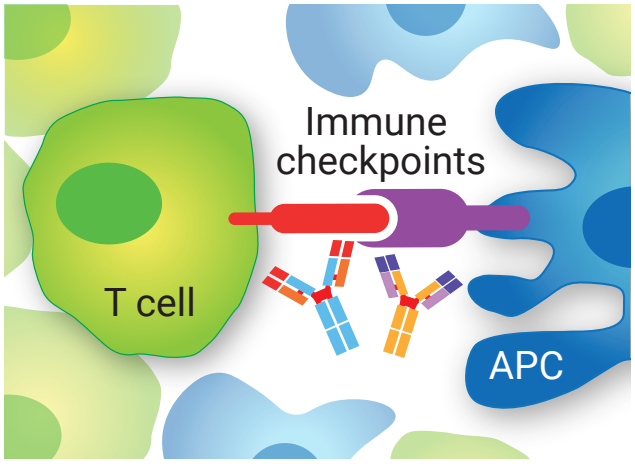
# IMMUNOTHERAPY

## IMMUNE CHECKPOINTS

## CHECKMATE ON CANCER

- *In vivo* validated mouse anti-mouse immune checkpoint (IC) mAbs
- Biosimilar IC mAbs with various IgG isotypes
- Functionally validated IC-expressing cells

The groundbreaking development of immune checkpoint (IC) blockade therapy with the use of monoclonal antibodies (mAbs) against PD-1, PD-L1, or CTLA-4, has revolutionized cancer treatment. InvivoGen provides an expanding collection of IC-related tools, such as mAbs and IC-expressing cell lines, to help you tackle the next big questions in the immuno-oncology research field.




WWW.INVIVOGEN.COM/IMMUNE-CHECKPOINTS

InvivoGen

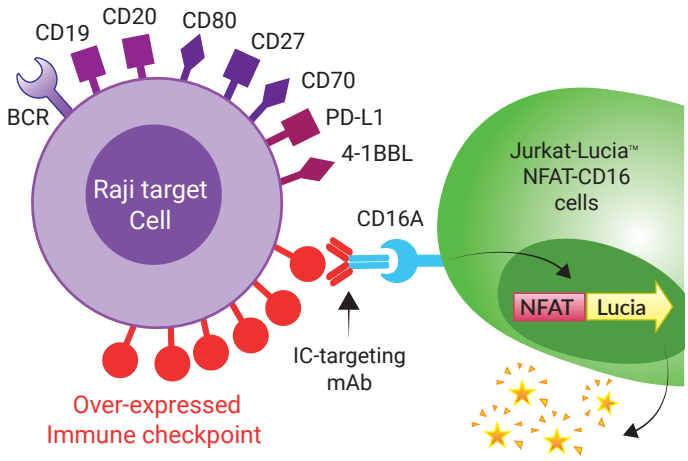
# Immune checkpoints: the stars of cancer treatment

Over the last decade, the understanding of the regulation of T cell responses has led to the groundbreaking and Nobel prize-winning development of immune checkpoint (IC) blockade. The discovery of ICs has revolutionized cancer treatment, however, their success in the clinic is still limited. In addition to the original ICs (PD-1, PD-L1, and CTLA-4), numerous other co-inhibitory (e.g. VISTA) and co-stimulatory (e.g. 4-1BB) ICs found on different cell types in the tumor microenvironment (TME) are being investigated. Novel immunotherapeutic approaches are targeting these ICs in combination with other targets to boost the immune response in the TME and enhance the efficiency of treatment. At InvivoGen, our diverse and technical expertise has allowed us to create innovative reagents and assays to support your research on ICs. We provide high-quality and functionally validated tools such as mouse anti-mouse monoclonal antibodies (mAbs) designed specifically for in vivo purposes (see back page) and an ADCC cell-based assay.

## IC-expressing Raji cells

InvivoGen provides an expanding **collection of Raji cells** that stably express ICs. These human B lymphocyte-derived cells are characterized by a number of surface-expressed markers including the B cell receptor (BCR), CD19, and CD20. Additionally, they naturally express various ICs such as CD27, CD70, CD80, and low levels of PD-L1 and 4-1BBL.

These cells can be used as target cells in human mAb effector studies such as **antibody-dependent cellular cytotoxicity** (ADCC) or **Antibody-dependent cellular phagocytosis** (ADCP), either with peripheral blood mononuclear cells, natural killer cells, or T lymphocyte cells, including Jurkat-Lucia™ NFAT-CD16 cells developed by InvivoGen (*see next page*).
InvivoGen's Raji cell collection is an integral part of our in-house quality control for our anti-human biosimilar IC mAbs.




InvivoGen's IC-expressing Raji target cells can be used in an ADCC assay using an IC-targeting mAb and our NFAT reporter T cell line.

| CELL LINE | PRODUCT | DESCRIPTION | REPORTER | CAT. CODE |
|---|---|---|---|---|
| IMMUNE CHECKPOINT TARGET CELLS | | | | |
| Raji (human) | Raji-Null | Human lymphoblast CD19/CD20/Control Target Cells | None | raji-null |
| | Raji-h4-1BB | Human lymphoblast h4-1BB expressing Cells | None | raji-h41bb |
| | Raji-hCTLA4 | Human lymphoblast hCTLA4 expressing Cells | None | raji-hctla4 |
| | Raji-HER2 | Human lymphoblast HER2 expressing Cells | None | raji-her2 |
| | Raji-hEGFR | Human lymphoblast hEGFR expressing Cells | None | raji-hegfr |
| | Raji-hICOS | Human lymphoblast hICOS expressing Cells | None | raji-hicos |
| | Raji-hLAG3 | Human lymphoblast hLAG3 expressing Cells | None | raji-hlag3 |
| | Raji-hOX40 | Human lymphoblast hOX40 expressing Cells | None | raji-hox40 |
| | Raji-hPD-1 | Human lymphoblast hPD-1 expressing Cells | None | raji-hpd1 |
| | Raji-hPD-L1 | Human lymphoblast hPD-L1 expressing Cells | None | raji-hpdl1 |
| | Raji-hTIGIT | Human lymphoblast hTIGIT expressing Cells | None | raji-htigit |
| | Raji-hVISTA | Human lymphoblast hVISTA expressing Cells | None | raji-hvista |

## Anti-PD-1/PD-L1 Cell-based Assay

InvivoGen provides **a paired cell line-based** assay to screen antibody-, Fc-fusion protein-, or small molecule-based inhibitors of the PD-1/PD-L1 axis. This assay is comprised of a TCR (T cell receptor)-expressing Jurkat reporter cell line (**Jurkat-Lucia TCR-hPD-1**) and an APC (antigen-presenting cell) Raji cell line (**Raji-APC-hPD-L1**). *An activating control APC Raji cell line (Raji-APC-Null) is also available.*

| CELL LINE | PRODUCT | DESCRIPTION | REPORTER | CAT. CODE |
|---|---|---|---|---|
| ANTI-PD-1/PD-L1 CELL-BASED ASSAY | | | | |
| PD-1/PD-L1 BioIC™ (kit) | Jurkat-Lucia™ TCR-hPD-1 + Raji-APC-hPD-L1 Cells | Effector and target cells | Lucia (Effector cells) | rajkt-hpd1 |
| Raji-APC-Null Cells | Control APC for PD-1/PD-L1 BioIC | Control target cells | None | raji-apc-null |

# Biosimilar IC mAb isotype families

To meet your needs, InvivoGen offers a growing number of biosimilar mAbs targeting various ICs. InvivoGen's biosimilar mAbs are research use only products that are highly similar to their clinically approved mAb counterpart. They feature the isotype used in the clinic (highlighted boxes) as well as various other isotypes, either natural or engineered to confer modified effector functions (e.g. antibody dependent cellular cytotoxicity (ADCC)).

**Natural isotype:**

- **IgG1** - High ADCC - ADCP and CDC

**Engineered isotypes (for modified effector functions):**

- **IgG1NQ or IgG1 (N298A)** - No ADCC due to mutations in the glycosylation sites of the CH2 region (non-glycosylated)
- **IgG1fut** - Enhanced ADCC due to defucosylation of the glycan sequences (non-fucosylated)
- **IgG4 (S228P)** - Low ADCC and includes the commonly used mutation in the hinge region preventing Fab arm exchanges

## WHY BUY YOUR IC MABS FROM INVIVOGEN?

- Recombinant (batch-to-batch consistency)
- High quality and sterile filtered
- Functionally validated (FACS and ADCC assay)
- Bulk sizes available upon request
- **Isotype controls available (Anti-β-Gal mAbs)**

| Specificity \ Isotype | hIgG1<br>High ADCC | hIgG1NQ<br>No ADCC | hIgG1fut<br>Enhanced ADCC | hIgG4 (S228P)<br>Low ADCC | QTY |
|---|---|---|---|---|---|
| Anti-hCTLA4 (similiar to Ipilimumab) | hctla4-mab1 | hctla4-mab12 | hctla4-mab13 | hctla4-mab14 | 100 µg |
| Anti-hPD-1 (similiar to Nivolumab) | hpd1ni-mab1 | hpd1ni-mab12 | hpd1ni-mab13 | hpd1ni-mab114 | 100 µg |
| Anti-hPD-1 (similiar to Pembrolizumab) | hpd1pe-mab1 | hpd1pe-mab12 | - | hpd1pe-mab14 | 100 µg |
| Anti-hPD-L1 (similiar to Atezolizumab) | hpdl1-mab1 | hpdl1-mab12 | hpdl1-mab13 | - | 100 µg |
| Anti-hTIGIT | htigit-mab1 | htigit-mab12 | - | - | 100 µg |
| Anti-hVISTA | hvista-mab1 | hvista-mab12 | hvista-mab13 | - | 100 µg |

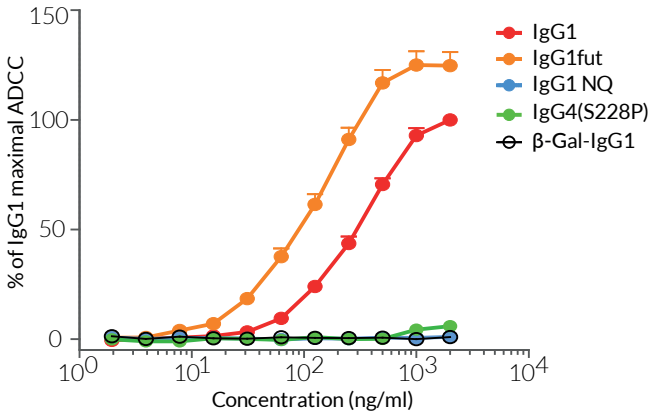
** Available in bulk quantity* ☐ *= isotype used in the clinic*

# ADCC & ADCP Assays

ADCC (antibody-dependent cellular cytotoxicity) and ADCP (antibody-dependent cell-mediated phagocytosis) are two major modes of action of therapeutic monoclonal antibodies (mAbs). InvivoGen offers a series of **Jurkat-Lucia™ NFAT cell lines**, specifically designed to assess the potency of specific immunoglobulin for ADCC and ADCP. These cells derive from the human T-lymphocyte Jurkat cell line and stably express CD16 or CD32, two Fc-gamma receptors (FcγR) for the constant region of immunoglobulins.

Jurkat cells naturally express a functional NFAT (nuclear factor of activated T cells) transcription factor, which is involved in the early signaling events of ADCC and ADCP. The level of ADCC/ADCP induction is measured as a bioluminescent signal produced by an NFAT-dependent Lucia luciferase reporter protein.

**Jurkat-Lucia™ NFAT-CD16** and **Jurkat-Lucia™ NFAT-CD32 cells** have been designed as effector reporter cells for InvivoGen's ADCC and ADCP assays, respectively. They can be used in combination with InvivoGen's Raji-derived Target Cells.




An example of an ADCC potency assay for natural and engineered isotypes of anti-human CTLA4 mAbs and a negative control mAb, β-Gal-IgG1, using InvivoGen's Jurkat-Lucia™ NFAT-CD16 Cells and Raji-hCTLA4 Cells.

| CELL LINE | PRODUCT | DESCRIPTION | REPORTER | CAT. CODE |
|---|---|---|---|---|
| ADCC AND ADCP EFFECTOR CELLS | | | | |
| Jurkat (human) | Jurkat-Lucia™ NFAT-CD16 | NFAT, human CD16A (FcγRIIIA, V158 allotype) transfected | Lucia | jktl-nfat-cd16 |
| | Jurkat-Lucia™ NFAT-CD32 | NFAT, human CD32A(FcgRIIA; H131 allotype) transfected | Lucia | jktl-nfat-cd32 |

# Mouse anti-mouse immune checkpoint mAbs

InvivoGen is developing a growing collection of recombinant mouse anti-mouse monoclonal antibodies (mAbs) for *in vivo* use, some of which are targeting the **immune checkpoints (ICs)** PD-1, PD-L1, and CTLA-4. These mAbs have been specifically engineered to limit their immunogenicity to maintain constant efficacy upon repeated injections in mice.

## Key Features of InvivoGen's InvivoFit™ IC mAbs

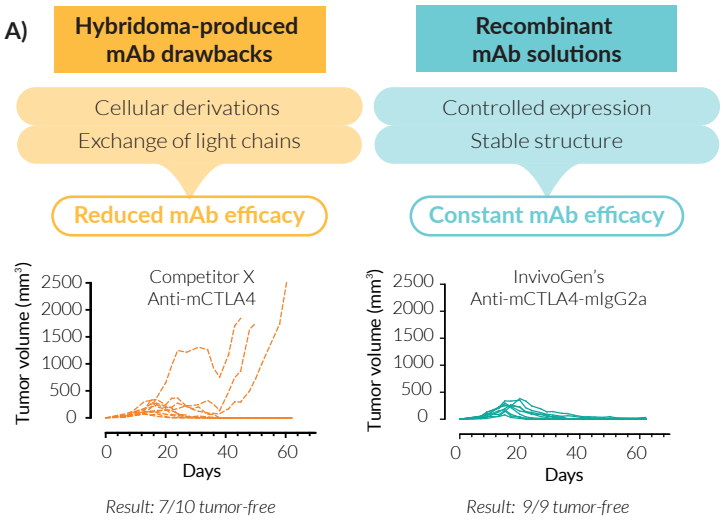
- mAb sequence is 85% to 100% murine
- Filter-sterilized (0.2 µm), with an endotoxin level <1 EU/mg
- Suitable for parenteral delivery in mice (azide-free)
- Low aggregation < 5%
- Produced in animal-free facilities with serum free media
- Functionally validated in mouse tumor models in vivo
- Isotype controls available (Anti-β-Gal mAbs)

## Why recombinant?

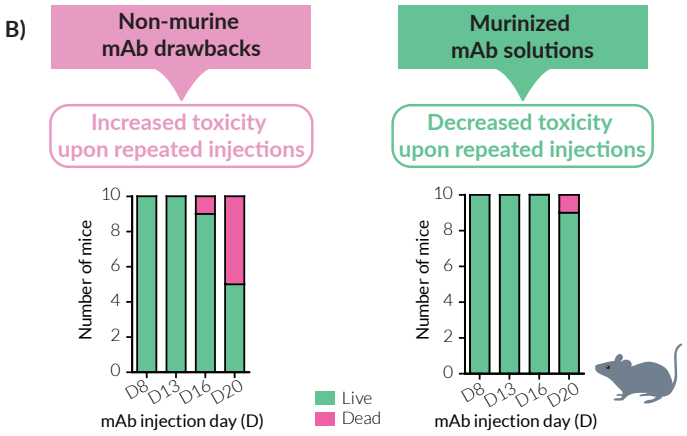
Unlike most IC mAbs on the market that are produced in hybridomas, InvivoGen's recombinant mouse anti-mouse IC mAbs are expressed and **produced in CHO cells** (virus-free status confirmed), ensuring structural stablity. Production of mAbs recombinantly guarantees the highest purity and batch-to-batch consistency (**Fig A**). They are provided in an **InvivoFit™ grade**, a high-quality standard specifically adapted for in vivo studies.

## Why use murinized mAbs in vivo?

Two major pitfalls encountered in murine in vivo studies upon repeated mAb injections are: *1) decreased antibody performance* and, *2) anti-species antibody generation* (if not of mouse origin). InvivoGen has limited these pitfalls for you by **murinizing the non-murine constant regions** of anti-human/rat IC mAbs with mouse IgG sequences for reduced toxicity in vivo (**Fig B**). Additionally, the isotype has been engineered for **optimal IC function**, for example InvivoGen's mIgG1e3 isotype contains a D265A mutation, resulting in the complete loss of cytolytic effector function.




**Benefit of recombinant mAbs in vivo:** BALB/c mice aged 10 weeks were challenged with 2 x 105 CT26 cells. After 8 days, Competitor X's Anti-mCTLA4 (hybridoma-produced) or InvivoGen's Anti-mCTLA4-mIg2a (recombinant) (200 µg/mouse) was administred intraparenterally in 200 µL sterile PBS into the mice. Tumor growth was monitored for 60 days.




**Importance of murinized mAbs in vivo:** 2 groups of 10 BALB/c mice aged 10 weeks were challenged with 2 x $10^5$ CT26 cells. After 8 days, Anti-hPD-L1-hIgG1 (non murine) or Anti-hPD-L1-mIgG1e3 (chimeric/murine) (200 µg/mouse) was administred intraparenterally in 200 µL sterile PBS into either group of mice. This was repeated on day 13, 16, and 20.

| PRODUCT | DESCRIPTION | QTY | CAT. CODE |
|---|---|---|---|
| Anti-mCTLA4-mIgG2a InvivoFit™ | 9D9-derived mouse mAb against murine CTLA-4 | 1 mg* | mctla4-mab10-1 |
| Anti-mPD-1-mIgG1e3 InvivoFit™ | RMP1-14-derived mouse mAb against murine PD-1 | 1 mg* | mpd1-mab15-1 |
| Anti-PD-L1-mIgG1e3 InvivoFit™ | Murinized atezolizumab mouse mAb against PD-L1 | 1 mg* | pdl1-mab15-1 |
| Anti-mTIGIT-mIgG2a InvivoFit™ | 10A7-derived monoclonal antibody against murine TIGIT | 1 mg* | mtigit-mab10-1 |
| Anti-β-Gal-mIgG1e3 InvivoFit™ | Mouse IgG1e3 isotype control | 1 mg* | bgal-mab15-1 |
| Anti-β-Gal-mIgG2a InvivoFit™ | Mouse IgG2a isotype control | 1 mg* | bgal-mab10-1 |

** Available in bulk and different quantities*

Mouse anti-mouse recombinant antibodies against lymphocyte markers or tumor-associated antigens are also available:

InvivoGen

Europe Tel: +33 562 71 69 39 Fax: +33 562 71 69 30 info.eu@invivogen.com
USA Tel: +1 888 457 5873 Fax: +1 858 457 5843 info@invivogen.com
Asia Tel: +852 3622 3480 Fax: +852 3622 3483 info.hk@invivogen.com