# Raji-hCTLA4 Cells

Human CTLA-4-expressing lymphoblast-like cells

Catalog code: raji-hctla4

https://www.invivogen.com/raji-hctla4

For research use only

Version 21B12-ED

## PRODUCT INFORMATION

### Contents and Storage

- 3-7 x $10^6$ Raji-hCTLA4 cells in a cryovial or shipping flask

*IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.*

- **1 ml of Blasticidin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

*Note: Data sheets for all components are available on our website.*

### Handling of Frozen Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

*Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.*

**IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.**

### Cell Line Stability

Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. To ensure maximum efficiency, do not passage Raji-Null cells more than 20 times and maintain cells in growth medium supplemented with the selective antibiotic.

### Quality Control

- Expression of human CTLA-4 has been verified by flow-cytometry.
- Induction of antibody-dependent cellular cytotoxicity (ADCC) has been validated using InvivoGen's anti-hCTLA4-hIgG1 antibody and Jurkat-NFAT Lucia™ CD16 reporter cell line.
- The stability for 20 passages following thawing has been verified.
- Raji-hCTLA4 cells are guaranteed mycoplasma-free.

## BACKGROUND

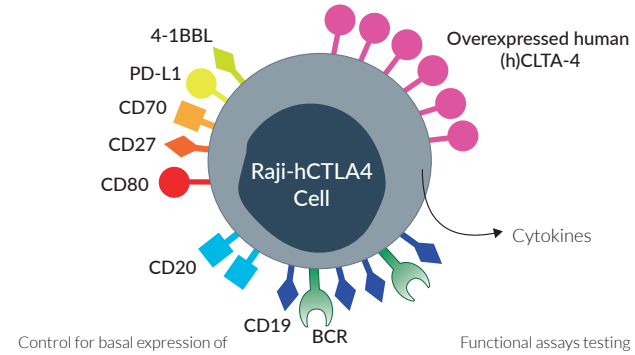
CTLA-4 (cytotoxic T cell lymphocyte antigen-4; also known as CD152) is a type I transmembrane protein expressed at the cell surface of activated conventional T cells, and constitutively on immunosuppressive regulatory T cells (Tregs)[1]. CTLA-4 is an inhibitory immune checkpoint that prevents T-cell overstimulation and host damage. It exerts competitive binding for stimulatory CD28 ligands (CD80/CD86). Ipilimumab, an anti-CTLA4 IgG1 monoclonal antibody (mAb) has been used to treat metastatic melanoma[1,2].

1. Ribas A. and Wolchock J.D., 2018. Cancer immunotherapy using checkpoint blockade. Science. 359:1350-55. 2. Almagro J.C. *et al.*, 2018. Progress and challenges in the development of antibodies for cancer therapy. Front. Immunol. 8:1751.

## PRODUCT DESCRIPTION

Raji-hCTLA4 cells were developed from the Raji cell line, a human B lymphocyte-derived cell line, as target cells for antibody-dependent cellular cytotoxicity (ADCC) assays. These cells have been successfully used as target cells in CAR-T cell toxicity assays as well as human effector studies such as ADCC, either with peripheral blood mononuclear cells, NK cells, or Jurkat-derived reporter T cells. Raji-hCTLA4 cells were stably transfected to express the human CTLA-4. Raji-hCTLA4 cells are characterized by a number of cell-surface expressed markers including the B cell receptor (BCR), CD19, and CD20. Additionally, they natrually express various ICs including CD27, CD70, CD80, and lower levels of PD-L1 and 4-1BBL. Raji-hCTLA4 cells can be used as target cells in ADCC assays using anti-human CTLA4 mAbs. These cells are selectable with blasticidin, which allows them to be used with the same selection pressure as other Raji-derived target cells from InvivoGen's collection

*Note: For more information, visit https://www.invivogen.com/raji-derived-target-cells*




*Possible applications with the Raji-hCTLA4 cell line.*

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FAQ Any questions about our cell lines? Visit our FAQ page.

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## SAFETY CONSIDERATIONS

**Biosafety Level 2**

Raji-hCTLA4 cells were derived from Raji cells, which contain Herpesvirus (EBV), and thus may require Biosafety Level 2. The biosafety level varies by country. Please check with your country's regulatory authority regarding the use of these cells.

## HANDLING PROCEDURES

**Required Cell Culture Medium**

- **Growth Medium:** IMDM, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™
- **Freezing Medium:** 90% FBS, 10% DMSO
- **Test Medium:** IMDM, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin and Blasticidin**

**Required Selective Antibiotic**

- Blasticidin

**Initial Culture Procedure**

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing must be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

*Note: All steps from this point should be carried out under strict aseptic conditions.*

3. Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge cells at 150 x g (RCF) for 10 mins.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% $CO_2$.

**Frozen Stock Preparation**

1. Resuspend cells at a density of 5-7 x $10^6$ cells/ml in freezing medium freshly prepared with cold FBS.
2. Aliquot 1 ml cells into cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long-term storage.

*Note: If properly stored, cells should remain stable for years.*

**Cell Maintenance**

1. After cells have recovered, subculture in growth medium with an initial seeding density of ~300,000 cells/ml. To maintain selection pressure, add 10 µg/ml of Blasticidin to the growth medium every passage.
2. Renew growth medium twice a week.

**Cell-Handling Recommendations**

To ensure the best results:

- Use Raji-hCTLA4 cells with less than 20 passages.
- Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% $CO_2$.

## APPLICATION

Raji-hCTLA4 cells have been designed as target cells for an antibody-dependent cellular cytotoxicity (ADCC) reporter assay using InvivoGen's Jurkat-Lucia™ NFAT CD16 cells, an immortalized T lymphocyte cell line that stably expresses the cell surface receptor CD16A (FcγRIIIA, V158 high affinity allotype), and an NFAT-inducible Lucia luciferase reporter gene.

For more information, visit https://www.invivogen.com/jurkat-lucia-nfat-cd16-cells.

*Note: Raji-Null cells can be used as negative target cell control.*

## ADCC REPORTER ASSAYS

**Cell Preparation**

1. Centrifuge Raji-hCTLA4 cells at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
2. Remove supernatant and resuspend Raji-hCTLA4 cells at 1.1 x $10^6$ cells/ml in fresh, pre-warmed test medium.

*Note: In steps 3 & 4, Jurkat-Lucia™ NFAT CD16 cells should be prepared just prior to their addition to the antibody-coated target cells.*

3. Centrifuge Jurkat-Lucia™ NFAT CD16 cells at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
4. Remove supernatant and resuspend Jurkat-Lucia™ NFAT CD16 cells at 2.2 x $10^6$ cells/ml in fresh, pre-warmed test medium.

*Important: To ensure reproducible results, homogenize the cell suspensions.*

**ADCC Induction**

Below is a protocol for end-point readings using a luminometer. This protocol can be adapted for use with kinetic measurements.

1. Add 20 µl of test anti-hCTLA4 mAb per well including a positive control (e.g. Anti-hCTLA4-hIgG1) and a negative control (e.g. Anti-β-Gal-hIgG1).

*Note: We recommend to prepare 1:4 or 1:2 dilution series.*

2. Add 90 µl of Raji-hCTLA4 cell suspension (~100,000 cells) per well of a flat-bottom 96-well plate.
3. Incubate the plate at 37 °C in a $CO_2$ incubator for 1 h.
4. Add 90 µl of Jurkat-Lucia™ NFAT CD16 cell suspension (~200,000 cells) per well.
5. Incubate the plate at 37 °C in a $CO_2$ incubator for 6 h.
6. Prepare QUANTI-Luc™ following the instructions on the data sheet.
7. Transfer 20 µl of co-incubated Raji-hCTLA4 and Jurkat-Lucia™ NFAT CD16 cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
8. Add 50 µl of QUANTI-Luc™ per well.
9. Proceed **immediately** with the measurement.

## RESTRICTIONS

**These cells are distributed for research purposes only.** This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

## RELATED PRODUCTS

| Product | Description | Cat. Code |
|---|---|---|
| Anti-β-Gal-hIgG1 | Control antibody | bgal-mab1 |
| Anti-hCTLA4-hIgG1 | Anti-hCTLA4 antibody | hctla4-mab1 |
| Blasticidin | Selection antibiotic | ant-bl-05 |
| Jurkat-Lucia™ NFAT-CD16 cells | ADCC reporter cell line | jktl-nfat-cd16 |
| QUANTI-Luc™ | Lucia detection medium | rep-qlc1 |
| Raji-Null cells | ADCC target cell line | raji-null |

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