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 19B04-NJ

PRODUCT INFORMATION

Contents and Storage

- **1 vial of Raji-hCTLA4 cells** (3-7 x 10^6 cells)

IMPORTANT: Cells are shipped frozen. If cells 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). Normocin™ is 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 Cells Upon Receipt

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: **Do not freeze the 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.

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

- Human CTLA-4 expression 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.

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.

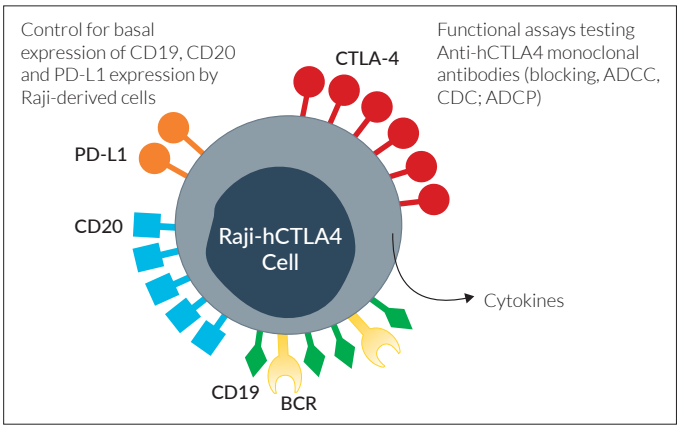
1. Ribas A. and Wolchock J.D., 2018. Cancer immunotherapy using checkpoint blockade. Science. 359:1350-55. 2. Almagro J.C. *et al.*, 2018. Progress and challenges in the development of antibodies for cancer therapy. Front. Immunol. 8:1751.

INTRODUCTION

Raji lymphoblast-like cells were established from a Burkitt's lymphoma patient. These cells have been successfully used as target cells in human effector studies such as antibody-dependent cellular cytotoxicity (ADCC), either with peripheral blood mononuclear cells, Natural Killer cells, or Jurkat-derived reporter T cells. 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].

PRODUCT DESCRIPTION

Raji-hCTLA4 cells were developed from the Raji cell line as target cells for ADCC assays. They were stably transfected to express the human CTLA-4 and blasticidin resistance genes. Raji-hCTLA4 cells constitutively express the CD19 and CD20 antigens, and low levels of PD-L1, an inhibitory checkpoint molecule. Raji-hCTLA4 cells can be used as target cells in ADCC assays using anti-human CTLA-4 mAbs. Their resistance to blasticidin allows them to be used with the same selection pressure than other Raji-derived target cells from InvivoGen's collection (For more information, visit https//www.invivogen.com/raji-derived -target-cells).



Examples of applications using the Raji-hCTLA4 cell line.

TECHNICAL SUPPORT
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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), 10 µg/ml Blasticicin, Pen-Strep (100 U/ml-100 µg/ml)
- **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 vial at 800 RPM (RCF = 150 g) for 7 minutes.
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 other 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 800 RPM (RCF 150 g) for 7 minutes.
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 800 RPM (RCF 150 g) for 7 minutes.
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.

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
Raji-hPD-1 cells	ADCC target cell line	raji-hpd1
Raji-hPD-L1 cells	ADCC target cell line	raji-hpdl1

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