Raji-hOX40 Cells

Human OX40-expressing lymphoblast-like cells

Catalog code: raji-hox40

https://www.invivogen.com/raji-hox40

For research use only

Version 21A19-ED

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 Raji-hOX40 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). 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 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-hOX40 cells more than 20 times and maintain cells in growth medium supplemented with the selective antibiotic.

Quality Control

- Expression of human OX40 has been verified by flow-cytometry.
- Induction of antibody-dependent cellular cytotoxicity (ADCC) has been validated using an anti-hOX40-hIgG1 antibody and InvivoGen's Jurkat-NFAT Lucia™ CD16 reporter cell line.
- The stability for 20 passages following thawing has been verified.
- Raji-hOX40 cells are guaranteed mycoplasma-free.

BACKGROUND

OX40 (also known as CD134, TNFRSF4) is a co-stimulatory immune checkpoint (IC) and a member of the tumor necrosis factor receptor superfamily (TNFRSF). OX40 is transiently expressed on activated CD4+ and CD8+ T-cells, while its ligand, OX40 ligand (OX40L, CD252, TNFSF4), is predominantly expressed on activated antigen presenting cells[1]. The engagement of OX40 with OX40L ultimately results in the activation of the NF-κB pathway[1,2].

The OX40-OX40L axis promotes the survival of effector T cells and the generation of T cell memory, while also being associated with local inflammation and auto-immune disease (e.g. SLE)[1,3]. For the latter, novel interventions that aim to antagonise this interaction are of interest in the clinic[3]. On the other hand, treatment with agonistic anti-OX40 mAbs has been shown to augment T cell differentiation and cytolytic function leading to enhanced anti-tumor immunity against a variety of tumor models[2]. Thus, combination therapies with other IC mAbs (e.g. anti-CTLA-4 and anti-PD-1) are being explored[2,4].

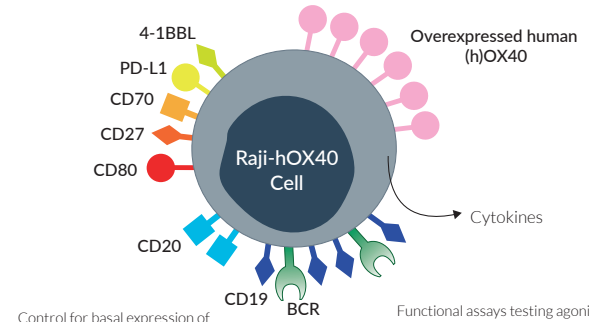
1. Gough, M.J. *et al.* 2009. OX40 (CD134) and OX40L. Adv Exp Med Biol 647, 94-107. **2. Alves Costa Silva, C. *et al.* 2020.** New pathways in immune stimulation: targeting OX40. ESMO Open, 5. **3. Webb, G.J. *et al.* 2016.** OX40, OX40L and Autoimmunity: a Comprehensive Review. Clin Rev Allergy Immunol 50, 312-332. **4. Linch, S.N. *et al.* 2015.** OX40 Agonists and Combination Immunotherapy: Putting the Pedal to the Metal. Front Oncol 5, 34.

PRODUCT DESCRIPTION

Raji-hOX40 cells were developed from the Raji cell line as target cells for ADCC assays. Raji lymphoblast-like cells were established from a Burkitt's lymphoma patient. These cells have been successfully used as target cells in CAR-T cell toxicity assays as well as human effector studies such as antibody-dependent cellular cytotoxicity (ADCC), either with peripheral blood mononuclear cells, natural killer (NK) cells, or Jurkat-derived reporter T cells.

Raji-hOX40 cells were stably transfected to overexpress the human (h)OX40 gene. Raji-hOX40 cells are characterized by a number of cell-surface expressed markers including the B cell receptor (BCR), CD19, and CD20. Additionally, they naturally express various ICs including CD27, CD70, CD80, and lower levels of PD-L1 and 4-1BBL. Raji-hOX40 cells can be used as target cells in ADCC assays using anti-human OX40 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: More information at https://www.invivogen.com/raji-derived-target-cells



TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

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

SAFETY CONSIDERATIONS

Biosafety Level 2

Raji-hOX40 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 vial at 150 x g (RCF) for 10 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 passage.
2. Renew growth medium twice a week.

Cell-Handling Recommendations

To ensure the best results:

- Use Raji-hOX40 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-hOX40 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.

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

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

ADCC REPORTER ASSAYS

Cell Preparation

1. Centrifuge the Raji-hOX40 cells at 150 x g (RCF) for 10 minutes or 300 x g (RCF) for 5 minutes.
2. Remove supernatant and resuspend Raji-hOX40 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 minutes or 300 x g (RCF) for 5 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-hOX40 mAb per well including a positive control 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-hOX40 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-hOX40 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
Blasticidin	Selection antibiotic	ant-bl-05
Jurkat-Lucia™ NFAT-CD16	ADCC reporter cell line	jktl-nfat-cd16
Normocin™	Anti-microbial agent	ant-nr-1
QUANTI-Luc™	Lucia detection medium	rep-qlc1
Raji-Null cells	ADCC target cell line	raji-null