Raji-HER2 Cells

HER2-expressing lymphoblast-like cells

Catalog code: raji-her2

https://www.invivogen.com/raji-her2

For research use only

Version 21B17-ED

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 Raji-HER2 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-HER2 cells more than 20 times and maintain cells in growth medium supplemented with the selective antibiotic.

Quality Control

- Expression of human HER2 has been verified by flow-cytometry.
- Induction of antibody-dependent cellular cytotoxicity (ADCC) has been validated using an anti-HER2-hIgG1 antibody and InvivoGen's Jurkat-NFAT Lucia™ CD16 reporter cell line.
- The stability for 20 passages following thawing has been verified.
- Raji-HER2 cells are guaranteed mycoplasma-free.

BACKGROUND

Human epidermal growth factor receptor-2 (HER2), also known as erbB2, is a member of the EGFR family of receptor tyrosine kinases. Due to its overexpression on the surface of not only breast cancer but a variety of solid tumors, HER2 is both an oncogene and a tumor-associated antigen[1,2]. Heterodimerization of HER2 with other members of the EGFR family results in the activation of a variety of potent proliferative and anti-apoptotic signaling pathways[1].

Therefore, HER2 is a major driver of tumor development and progression[1]. There are a variety of approved monoclonal antibody (mAb) therapies (e.g. Trastuzumab) successfully used in the treatment of HER2-positive cancers (e.g. breast and gastric)[2]. Thus, HER2 remains an important therapeutic target for the development of more potent mAbs, specifically with optimized effector functions for greater tumor-directed antibody-dependent cell cytotoxicity (ADCC)[2].

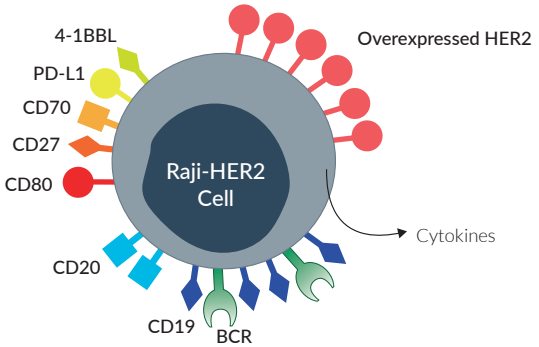
1. Gutierrez, C. & Schiff, R. 2011. HER2: biology, detection, and clinical implications. Arch Pathol Lab Med 135, 55-62. **2. Oh, D.Y. & Bang, Y.J. 2020.** HER2-targeted therapies - a role beyond breast cancer. Nat Rev Clin Oncol 17, 33-48.

PRODUCT DESCRIPTION

Raji-HER2 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, NK cells, or Jurkat-derived reporter T cells.

Raji-HER2 cells were stably transfected to overexpress the human *HER2* gene. Raji-HER2 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 immune checkpoints including CD27, CD70, CD80, and lower levels of PD-L1 and 4-1BBL. Raji-HER2 cells can be used as target cells in ADCC assays with anti-HER2 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



Control for basal expression of CD19, CD20 and naturally expressed immune checkpoints by Raji-derived cells

Functional assays testing Anti-HER2 monoclonal antibodies (ADCC, CDC, ADCP)

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

Raji-HER2 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), 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-HER2 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-HER2 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.

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

ADCC REPORTER ASSAYS

Cell Preparation

1. Centrifuge the Raji-HER2 cells at 150 x g (RCF) for 10 minutes or 300 x g (RCF) for 5 minutes.
2. Remove supernatant and resuspend 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-HER2 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-HER2 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-HER2 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