Jurkat-Lucia™ NFAT-CD16-Low Cells

NFAT-CD16-Low Lucia Luciferase Reporter T Lymphocytes

Catalog code: jktl-nfat-cd16lo

https://www.invivogen.com/jurkat-lucia-nfat-cd16-cells

For research use only

Version 23J25-AK

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of Jurkat-Lucia™ NFAT-CD16-Low 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 Hygromycin B Gold** (100 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Zeocin®** (100 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.

- **1 tube of QUANTI-Luc™ 4 Reagent**, a lucia luciferase detection reagent (sufficient to prepare 25 ml). Store at -20 °C. Avoid repeated freeze-thaw cycles.

Note: QUANTI-Luc™ 4 Reagent is photosensitive and should be protected from light.

Handling 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'.

Quality Control

- Human CD16A expression has been verified by flow-cytometry.
- Induction of antibody-dependent cellular cytotoxicity (ADCC) has been validated using InvivoGen's Anti-hCD20-hIgG1 antibody and Raji-Null target cell line.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

Cell Line Stability

Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

PRODUCT DESCRIPTION

InvivoGen offers Jurkat-Lucia™ NFAT-CD16-Low cells, specifically designed to assess the potency of specific immunoglobulin for ADCC (antibody-dependent cellular cytotoxicity).

Jurkat-Lucia™ NFAT-CD16-Low cells were engineered from the human T-lymphocyte Jurkat cell line. Jurkat cells naturally express a functional NFAT pathway[4]. Jurkat-Lucia™ NFAT-CD16-Low cells stably express the cell surface Fc receptor CD16A (FcγRIIIA; F158 allotype [2,3]) and the Lucia luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements. These cells have been functionally tested with the Raji-Null target cell line and Anti-hCD20 IgG isotypes. Antibodies displaying lower EC_{50} have higher ADCC potency.

These cells are selectable to Blasticidin, Hygromycin, and Zeocin®.

BACKGROUND

Antibody-dependent cellular cytotoxicity (ADCC) is an immune mechanism through which Fc receptor-bearing effector cells can recognize and kill antibody (Ab)-coated target cells expressing antigens on their surface. ADCC is triggered by the cross-linking between antigen-bound Abs and the Fc receptor CD16A (FcγRIIIA) at the surface of immune effector cells, such as Natural Killer cells. These interactions induce the increase of intracellular calcium concentrations and the translocation of the NFAT transcription factor to the nucleus, where it can bind to the promoter regions of ADCC relevant genes[1].

CD16A features allelic polymorphisms among the human population, notably at position 158 in the mature protein (or position 161 in the full protein)[2]. The F158 allotype is reported to have lower affinity for monoclonal immunoglobulin G (IgGs) than the V158 allotype [2,3].

1. Leibson P.J., 1997. Signal transduction during natural killer cell activation: inside the mind of a killer. Immunity. 6:655. **2. Bruhns P. *et al.*, 2009.** Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses. Blood. 113(16):3716. **3. Nagelkerke S.Q. *et al.*, 2019.** Genetic variation in low-to-medium-affinity Fcγ receptors: functional consequences, disease associations, and opportunities for personalized medicine. Front. Immunol. 10:2237. **4. Shaw J-P. *et al.*, 1998.** Identification of a putative regulator of early T cell activation genes. Science. 241:202.

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.

SAFETY CONSIDERATIONS

Biosafety Level 1

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), 100 µg/ml Normocin™, 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, Blasticidin, Hygromycin B Gold, and Zeocin®**

Required Selective Antibiotics

- Blasticidin, Hygromycin B Gold, and Zeocin®

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 min.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium.
6. Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium **without selective antibiotics**.
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. Jurkat-Lucia™ NFAT-CD16-Low cells grow in suspension.
2. After cells have recovered (after at least one passage), maintain and subculture the cells in growth medium. To maintain selection pressure, add 5 µg/ml of Blasticidin, 50 µg/ml of Hygromycin B Gold, and 100 µg/ml of Zeocin® to the growth medium every other passage.
3. Pass the cells every 3 days by inoculating 2-5 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Note: The average doubling time for the Jurkat-Lucia™ NFAT-CD16-Low cells is ~ 48 hours using the conditions described above.

Cell-Handling Recommendations

To ensure the best results, use Jurkat-Lucia™ NFAT-CD16-Low cells with less than 20 passages.

APPLICATION

Jurkat-Lucia™ NFAT-CD16-Low cells have been designed as effector reporter cells for InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using our expanding collection of Raji-derived target cells. For more information, visit www.invivogen.com/raji-derived-target-cells.

ADCC REPORTER ASSAYS

Below is a protocol to perform an ADCC assay with Raji-Null cells which constitutively express human CD20 at the cell surface.

Cell Preparation

Pass effector and target cells 2 days prior to the reporter assay.

1. Day -2: Resuspend Jurkat-Lucia™ NFAT-CD16-Low cells at 5 x 10^5 cells/ml, and Raji-Null cells at 4 x 10^5 cells/ml in pre-warmed test medium.
2. Incubate at 37 °C in a CO_2 incubator for 48 h.
3. Day 0: Centrifuge Raji-Null cells at 300 x g (RCF) for 5 min.
4. Remove supernatant and resuspend at 1.1 x 10^6 cells/ml in pre-warmed test medium.

Note: In steps 5 & 6, Jurkat-Lucia™ NFAT-CD16-Low cells should be prepared just prior to their addition to the antibody-coated target cells.

5. Centrifuge Jurkat-Lucia™ NFAT-CD16-Low cells at 300 x g (RCF) for 5 min.
6. Remove supernatant and resuspend 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-hCD20 mAb per well including a positive control (e.g. Anti-hCD20 IgG1) and a negative control (e.g. Anti-β–galactosidase IgG1).

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

2. Add 90 µl of Raji-Null 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-Low 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™ 4 Reagent working solution following the instructions on the data sheet.
7. Transfer 20 µl of co-incubated Raji-Null and Jurkat-Lucia™ NFAT-CD16-Low cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
8. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution per well.
9. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Anti-β-Gal-hIgG1	Control antibody	bgal-mab1
Anti-hCD20-hIgG1	Anti-hCD20 antibody	hcd20-mab1
Blasticidin	Selection antibiotic	ant-bl-05
Hygromycin B Gold	Selection antibiotic	ant-hg-1
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
Raji-Null cells	ADCC target cell line	raji-null
Zeocin®	Selection antibiotic	ant-zn-05