Jurkat-Lucia™ NFAT-CD16 Cells

NFAT-CD16 Lucia Luciferase Reporter T Lymphocytes
Catalog code: jktl-nfat-cd16
https://www.invivogen.com/jurkat-lucia-nfat-cd16-cells

For research use only
Version 19B04-NJ

PRODUCT INFORMATION

Contents and Storage

- **1 vial of Jurkat-Lucia™ NFAT-CD16 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 Zeocin™** (100 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.*
- **1 pouch of QUANTI-Luc™**. Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable 1 week at 4 °C or 1 month at -20 °C. Keep reconstituted QUANTI-Luc™ away from light.

*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 Jurkat-Lucia™ NFAT-CD16 cells more than 20 times and maintain cells in growth medium supplemented with the selective antibiotic.

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.
- Jurkat-Lucia™ NFAT-CD16 cells are guaranteed mycoplasma-free.

RESTRICTIONS

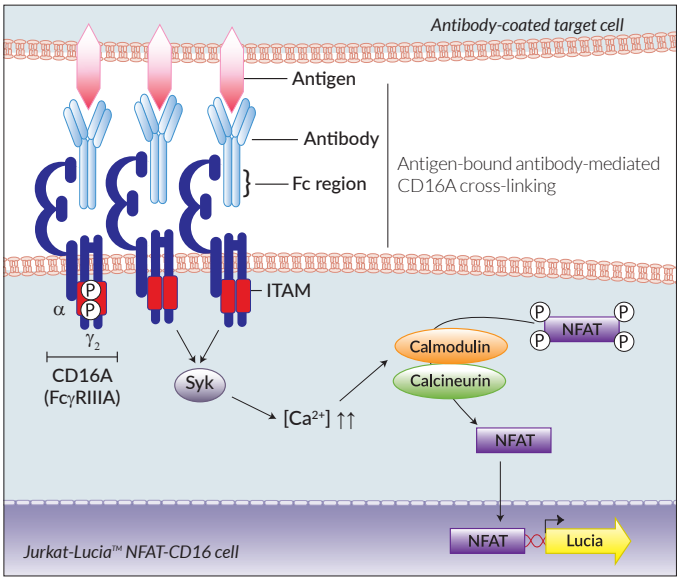
These cells are distributed for research purposes only. This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses.

INTRODUCTION

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].

PRODUCT DESCRIPTION

Jurkat-Lucia™ NFAT-CD16 cells were engineered from the human T-lymphocyte Jurkat cell line. Jurkat cells naturally express a functional NFAT pathway[2]. Jurkat-Lucia™ NFAT-CD16 cells stably express the cell surface Fc receptor CD16A (FcγRIIIA; V158 high affinity allotype[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 various target cells and specific monoclonal Ab isotype combinations. Antibodies displaying lower EC_{50} have higher ADCC potency. These cells are resistant to Blasticidin and Zeocin™.



1. Leibson P.J., 1997. Signal transduction during natural killer cell activaion: inside the mind of a killer. Immunity. 6:655-61. 2. Shaw J-P. *et al.*, 1998. Identification of a putative regulator of early T cell activation genes. Science. 241:202-205. 3. Quast I. *et al.*, 2016. Regulation of antibody effector functions through IgG Fc N-glycosylation. Cell. Mol. Life. Sci. 74(5):837-47.

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

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), 10 µg/ml Blasticicin, 100 µg/ml Zeocin™, 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, and Zeocin™**

Required Selective Antibiotics

- Blasticidin 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 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 to 400,000 cells/ml.To maintain selection pressure, add 10 µg/ml of Blasticidin and 100 µg/ml of Zeocin™ to the growth medium every other passage.
2. Renew growth medium twice a week.

Cell-Handling Recommendations

To ensure the best results:

- Use Jurkat-Lucia™ NFAT CD16 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

Jurkat-Lucia™ NFAT-CD16 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 (e.g. Raji-Null cells, Raji-hCTLA4 cells, Raji-hPD-1 cells, Raji-hPD-L1 cells) .

For more information, visit https://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

1. Centrifuge Raji-Null cells at 800 RPM (RCF 150 g) for 7 minutes.
2. Remove supernatant and resuspend Raji-Null 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-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 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-Null 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-hCD20-hIgG1	Anti-hCD20 antibody	hcd20-mab1
Blasticidin	Selection antibiotic	ant-bl-05
QUANTI-Luc™	Lucia detection medium	rep-qlc1
Raji-hCTLA4 cells	ADCC target cell line	raji-hCTLA4
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
Zeocin™	Selection antibiotic	ant-zn-05

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InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com