Jurkat-Lucia™ NFAT-CD28 Cells

NFAT-CD28 Lucia Luciferase Reporter T Lymphocytes

Catalog code: jktl-nfat-cd28

https://www.invivogen.com/jurkat-lucia-nfat-cd28-cells

For research use only

Version 23J25-AK

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of Jurkat-Lucia™ NFAT-CD28 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 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 CD28 expression has been verified by flow-cytometry.
- The activation of NFAT has been confirmed following CD3 and CD28 cross-linking, by measuring the levels of secreted Lucia luciferase.
- The stability for 20 passages following thawing has been verified.
- Jurkat-Lucia™ NFAT-CD28 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

Jurkat-Lucia™ NFAT-CD28 cells were engineered from Jurkat-Lucia™ NFAT cells, a human T-lymphocyte Jurkat cell line. Jurkat cells naturally express a functional NFAT pathway[4]. Jurkat-Lucia™ NFAT-CD28 cells stably express the Lucia luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements. They were obtained by stable transfection of Jurkat-Lucia™ NFAT cells with the gene encoding human CD28. NFAT activation can be readily measured as a bioluminescent signal produced by the Lucia luciferase using the detection reagent QUANTI-Luc™.

These cells are resistant to Blasticidin and Zeocin®.

BACKGROUND

NFAT (nuclear factor of activated T cells) proteins are a family of transcription factors involved in T cell activation, differentiation, and self-tolerance[1,2]. They regulate the expression of many genes, either alone, or in cooperation with other transcription factors[1,2].

CD28 is a transmembrane protein expressed by T cells. Its interaction with CD80 (B7-1) or CD86 (B7-2) on antigen-presenting cells (APCs), in conjunction with T cell receptor (TCR) engagement, triggers a co-stimulation signal[3]. Most NFAT proteins are controlled by calcium influx upon TCR stimulation. The co-engagement of CD28, triggers the activation of the AKT kinase, which contributes to enhancing NFAT translocation into the nucleus [2]. Several therapeutic approaches have focused on targeting the NFAT signaling to control T cell responses in autoimmune diseases and graft rejection[1].

1. Lee J-U., *et al.*, 2018. Revisiting the Concept of Targeting NFAT to Control T Cell Immunity and Autoimmune Diseases. Front Immunol. DOI: 10.3389/fimmu.2018.02747. **2. Macian F., 2005.** NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol. 5(6):472-484. **3. Smith-Garvin J.E. *et al.*, 2009.** T Cell Activation. Ann. Rev. Immunol. 27:591-619. **4. Shaw J-P. *et al.*, 1998.** Identification of a putative regulator of early T cell activation genes. Science. 241:202-205.

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.

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

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, 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 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.

Note: The average viability for the Jurkat-Lucia™ NFAT-CD28 cells is ~60% to 70% at 48 hours after thawing.

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-CD28 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 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-CD28 cells is ~48 hours using the conditions described above.

Cell-Handling Recommendations

To ensure the best results:

- Use Jurkat-Lucia™ NFAT-CD28 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-CD28 cells have been designed for the screening of NFAT-targeting drugs.

Below is a protocol for assessing NFAT activation upon cross-linking of CD3 and CD28 at the cell surface using monoclonal antibodies (mAbs).

REPORTER ASSAY

Cell Preparation

Pass Jurkat-Lucia™ NFAT-CD28 cells 2 days prior to the reporter assay:

1. Day -2: Centrifuge cells at 300 x g (RCF) for 5 min.
2. Remove supernatant and resuspend at 0.5 x 10^6 cells/ml in fresh, pre-warmed test medium.
3. Incubate at 37 °C in a CO_2 incubator for 48 h.
4. Day 0: Just prior to activation, prepare a suspension at 1.8 x 10^6 cells/ml in fresh, pre-warmed test medium.

IMPORTANT: To ensure reproducible results, homogenize the cell suspension.

NFAT 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 anti-hCD3 mAb (final concentration 0.5 µg/ml) and 20 µl of anti-hCD28 mAb (final concentration 0.5 µg/ml) per well of a flat-bottom 96-well plate.
2. Add 160 µl of Jurkat-Lucia™ NFAT-CD28 cell suspension (~300,000 cells) per well.
3. Incubate the plate at 37 °C in a CO_2 incubator for 6 h.
4. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
5. Transfer 20 µl of 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
Blasticidin	Selection antibiotic	ant-bl-05
Ionomycin	Calcium ionophore	inh-ion
Jurkat-Lucia™ NFAT	Reporter cell line	jktl-nfat
Normocin™	Antimicrobial agent	ant-nr-1
PMA	PKC/NF-κB activator	tlrl-pma
QUANTI-Luc™ 4 Lucia/Gaussia	Luminescence detection kit	rep-qlc4lg1
Zeocin®	Selection antibiotic	ant-zn-1