Jurkat-Lucia™ NFAT Cells

NFAT-Luc Reporter T lymphocytes

Catalog code: jktl-nfat

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

For research use only

Version 23J25-AK

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of Jurkat-Lucia™ NFAT 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 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.

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

- Data sheets for all components are available on our website.

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

- The activation of NFAT has been confirmed following the induction of Jurkat-Lucia™ NFAT cells with PMA/ionomycin, phytohaemagglutinin P, or concanavalin A by measuring the levels of Lucia luciferase secreted.
- The stability for 20 passages following thawing has been verified.
- Jurkat-Lucia™ NFAT 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 cells were engineered from the human T lymphocyte Jurkat cell line. Jurkat cells naturally express a functional NFAT pathway[3]. Jurkat-Lucia™ NFAT cells stably express the Lucia luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements. NFAT activation can be readily measured as a bioluminescent signal produced by the Lucia and Gaussia luciferase using the detection reagent QUANTI-Luc™ 4 Lucia/Gaussia™.

These cells are selectable with 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]. Most NFAT proteins are controlled by calcium influx upon T cell receptor (TCR) stimulation[1,2].

Calcium binds calmodulin, which in turn activates calcineurin, a calmodulin-dependent phosphatase. Calcineurin. dephosphorylates NFAT proteins, leading to their translocation into the nucleus, where they regulate the expression of many genes, either alone, or in cooperation with other transcription factors[1,2]. 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. 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.

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 and Zeocin®**

Required Selective Antibiotics

- 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 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 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.

Cell-Handling Recommendations

To ensure the best results:

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

REPORTER ASSAY

Below is a protocol using Jurkat-Lucia™ NFAT cells to screen for NFAT-targeting compounds. It is recommended to perform the assay with test medium that does not contain Normocin™ or Zeocin®.

Cell Preparation

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

1. Day -2: Resuspend Jurkat-Lucia™ NFAT cells at 5 x 10^5 cells/ml in fresh, pre-warmed test medium.
2. Incubate at 37 °C in a CO_2 incubator for 48 h.
3. Day 0: Centrifuge Jurkat-Lucia™ NFAT cells at 300 x g (RCF) for 5 min.
4. Remove supernatant and resuspend at 2 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 test sample per well of a flat-bottom 96-well plate.
2. Include PMA (50 ng/ml) + ionomycin (3 µg/ml), or concanavalin A (10 µg/ml) as a positive control, and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
3. Add 180 µl of Jurkat-Lucia™ NFAT cell suspension (~360,000 cells) per well.
4. Incubate the plate at 37 °C in a CO_2 incubator for 18-24 h.
5. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
6. Transfer 20 µl of cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
7. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution per well.
8. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Concanavalin A	NFAT activator	inh-cona
Ionomycin	Calcium ionophore	inh-ion
Jurkat-Lucia™ NFAT-CD28	Reporter cell line	jktl-nfat-cd28
Normocin™	Antimicrobial agent	ant-nr-1
PHA-P	NFAT activator	inh-phap
PMA	PKC/NF-κB activator	tlrl-pma
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1
Zeocin®	Selective antibiotic	ant-zn-1