

Jurkat-Lucia™ NFAT-CD28 Cells

NFAT-CD28 Lucia Luciferase Reporter T Lymphocytes

Catalog code: jktl-nfat-cd28

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

For research use only

Version 22C08-NJ

PRODUCT INFORMATION

Contents and Storage

- 1 vial of Jurkat-Lucia™ NFAT-CD28 cells (3-7 x 10⁶ cells)

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- 1 ml of Blastidicin (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.*
- 1 pouch of QUANTI-Luc™. Store QUANTI-Luc™ pouch at -20°C for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4°C or for 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 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.

INTRODUCTION

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 signal³. 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 nucleus². Several therapeutic approaches have focused on targeting the NFAT signaling to control T cell responses in autoimmune diseases and graft rejection¹.

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 pathway⁴. 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 Blastidicin and Zeocin®.

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.

TECHNICAL SUPPORT

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Any questions about our cell lines?

Visit our FAQ page.

InvivoGen
www.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, 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% CO₂.

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 10⁶ 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 10⁵ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ 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% CO₂.

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 10⁶ cells/ml in fresh, pre-warmed test medium.
3. Incubate at 37 °C in a CO₂ incubator for 48 h.
4. Day 0: Just prior to activation, prepare a suspension at 1.8 x 10⁶ 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 CO₂ incubator for 6 h.
4. Prepare [QUANTI-Luc™](#) 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™](#) 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™	Detection reagent	rep-qlc1
Zeocin®	Selection antibiotic	ant-zn-1

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QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2

<https://www.invivogen.com/quantiluc>

For research use only

Version 19A04-MM

PRODUCT INFORMATION

Contents

QUANTI-Luc™ is provided as packs of individually sealed pouches.

- rep-qlc1: 2 pouches of QUANTI-Luc™
- rep-qlc2: 5 pouches of QUANTI-Luc™

Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability

- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

DESCRIPTION

QUANTI-Luc™ is an assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS

Preparation of QUANTI-Luc™

1. Pour the pouch contents into a 50 ml screw cap tube.
2. Add 25 ml of sterile water.
3. Swirl product gently until powder is completely dissolved.
4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

Detection of luciferase activity from cell culture medium

To obtain **end-point readings** using a luminometer **with an injector**.

1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with the QUANTI-Luc™ assay solution and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.

1. Set the luminometer with a 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ assay solution to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™ Gold (For standard and HTS assays)	rep-qlcg1
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
Recombinant Lucia luciferase protein	rec-lucia
Reporter Cells	
THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-κB Cells	thp1-nfkb

For a complete list of InvivoGen's Lucia luciferase Reporter Cell Lines visit <https://www.invivogen.com/lucia-reporter-cells>.

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