Jurkat-Lucia™ NFAT-CD32 Cells

NFAT-CD32 Lucia Luciferase Reporter T Lymphocytes

Catalog code: jktl-nfat-cd32

https://www.invivogen.com/jurkat-lucia-nfat-cd32-cells

For research use only

Version 23J25-AK

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of Jurkat-Lucia™ NFAT-CD32 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 CD32A expression has been verified by flow-cytometry.
- Induction of antibody-dependent cellular phagocytosis (ADCP) has been validated using InvivoGen's Anti-hCD20-hIgG2 antibody and Raji-Null target cell line.
- The stability for 20 passages following thawing has been verified.
- Jurkat-Lucia™ NFAT-CD32 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-CD32 cells, specifically designed to assess the potency of specific immunoglobulin for ADCP (antibody-dependent cell-mediated phagocytosis).

Jurkat-Lucia™ NFAT-CD32 cells were engineered from the human T-lymphocyte Jurkat cell line. Jurkat cells naturally express a functional NFAT pathway[4]. Jurkat-Lucia™ NFAT-CD32 cells stably express the cell surface Fc receptor CD32A (FcγRIIA; H131 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.

These cells are selectable with Blasticidin and Zeocin®.

BACKGROUND

Antibody-dependent cell-mediated phagocytosis (ADCP) is an immune mechanism through which Fc receptor-bearing effector cells can recognize and clear antibody (Ab)-coated microbes or -target cells[1,2]. ADCP is triggered by the cross-linking between antigen-bound Abs and the Fc receptor CD32A (FcγRIIA) at the surface of monocytes, macrophages, and dendritic cells[1,2]. 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 ADCP relevant genes[1,2]. CD32A features allelic polymorphisms among the human population, notably at position 131 in the mature protein (or position 166 in the full protein)[3]. The H131 allotype is reported to have higher affinity for monoclonal immunoglobulin G (IgGs) than the R131 allotype[3].

1. Quast I. et al., 2016. Regulation of antibody effector functions through IgG Fc N-glycosylation. Cell. Mol. Life. Sci. 74(5):837-47. **2. Tay M.Z. *et al.*, 2019.** Antibody-Dependent Cellular Phagocytosis in Antiviral Immune Responses. Front Immunol. 10:332. **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.

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FAQ Any questions about our cell lines?
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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% 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-CD32 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 10 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-CD32 cells is ~ 48 hours using the conditions described above.

Cell-Handling Recommendations

To ensure the best results, use Jurkat-Lucia™ NFAT-CD32 cells with less than 20 passages.

APPLICATION

Jurkat-Lucia™ NFAT-CD32 cells have been designed as effector reporter cells for InvivoGen's antibody-dependent cell-mediated phagocytosis (ADCP) assay using our expanding collection of Raji-derived target cells. For more information, visit www.invivogen.com/raji-derived-target-cells.

ADCP REPORTER ASSAYS

Below is a protocol to perform an ADCP 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-CD32 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-CD32 cells should be prepared just prior to their addition to the antibody-coated target cells.

5. Centrifuge Jurkat-Lucia™ NFAT-CD32 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.

ADCP 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 IgG2) and a negative control (e.g. Anti-β–galactosidase IgG2).

Note: We recommend to prepare a 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-CD32 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-CD32 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-hIgG2	Control antibody	bgal-mab2
Anti-hCD20-hIgG2	Anti-hCD20 antibody	hcd20-mab2
Blasticidin	Selection antibiotic	ant-bl-05
QUANTI-Luc™ 4 Lucia/Gaussia	Luminescence detection kit	rep-qlc4lg1
Raji-Null cells	ADCC/P target cell line	raji-null
Zeocin®	Selection antibiotic	ant-zn-05

QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 23F27-AK

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia or Renilla kits.

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is one component of the QUANTI-Luc™ 4 Lucia/Gaussia and QUANTI-Luc™ 4 Renilla kits. It contains the coelenterazine substrate for the detection of secreted Lucia or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of Lucia luciferase activity in 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 QUANTI-Luc™ 4 Reagent 1X 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™ 4 Reagent 1X 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	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit comprising QUANTI-Luc™ 4 Reagent & Stabilizer	rep-qlc4lg1
QUANTI-Luc™ 4 Renilla Kit comprising QUANTI-Luc™ 4 Reagent & Lysis buffer	rep-qlc4r1

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