THP1-Dual™ KO-IRF3 Cells

IRF3 knockout NF-κB-SEAP & IRF-Lucia luciferase reporter monocytes

Catalog code: thpd-koirf3

https://www.invivogen.com/thp1-dual-koirf3

For research use only

Version 19K08-MM

PRODUCT INFORMATION

Contents

- **1 vial of THP1-Dual™ KO-IRF3 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 -20°C.*
- **1 ml of Zeocin™** (100 mg/ml). Store at 4°C or -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 pouch of QUANTI-Luc™** (Lucia luciferase detection reagent). Store pouch at -20°C. Reconstituted **QUANTI-Luc™** is stable for 1 week at 4°C and for 1 month at -20°C. Protect from light.
- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20°C. QUANTI-Blue™ Solution is stable for 2 weeks at 4°C and for 2 months at -20°C.

Note: Data sheets for all components are available on our website.

Handling 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.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness 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.

Quality Control

- Biallelic IRF3 knockout has been verified by PCR, DNA sequencing, Western blot, and functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

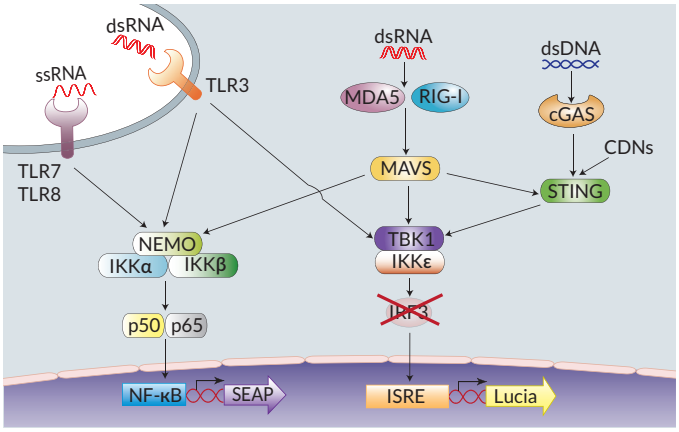
These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees with 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.

BACKGROUND

Several groups of pattern recognition receptors (PRRs) recognize the abnormal presence of nucleic acids (e.g. viral DNA or RNA) in the cytosol. These PRRs include the cytosolic DNA sensor (CDS), cyclic GMP-AMP synthase (cGAS), the cyclic dinucleotide sensor, stimulator of interferon genes (STING), the cytoplasmic RNA sensor, retinoic acid inducible gene I (RIG-I), and endosomal Toll-like receptors (TLRs)[1]. Upon ligand binding, these receptors trigger the production of interferons (IFNs) through the activation of the TBK1 (TANK-binding kinase 1)/IKKε-IRF3/IRF7 (interferon regulatory factors 3 and 7) pathway[1]. IRF3 and IFR7 bind to ISRE (IFN-stimulated response elements) in the promoters of ISGs (IFN-stimulated genes). PRRs detecting cytoplasmic nucleic acids also trigger the production of pro-inflammatory cytokines through the activation of NF-κB pathway[1].

1. Iurescia S. *et al.*, 2018. Nucleic acid sensing machinery: targeting innate immune system for cancer therapy. Recent Pat. Anticancer Drug Discov. 13: 2-17.



PRODUCT DESCRIPTION

THP1-Dual™ KO-IRF3 cells were generated from the THP1-Dual™ cell line, which is derived from the human THP-1 monocytes, through the stable knockout of the *IRF3* gene. These cells feature two reporter genes allowing the simultaneous study of the IRF pathway, by monitoring the activity of an inducible secreted Lucia luciferase, and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). Lucia luciferase and SEAP activities are readily assessable in the supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively. As expected, IRF and NF-κB responses are severely impaired when the cells are incubated with STING agonists, such as 2'3'-cGAMP, which do not require transfection to access the cytosol. However, we have observed different responses (either no effect, decrease or increase) when using RNA or DNA agonists with transfection reagents. THP1-Dual™ KO-IRF3 cells retain the ability to respond to cytokines such as type I IFNs and TNF-α. These cells are resistant to Blasticidin and Zeocin™.

TECHNICAL SUPPORT
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SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™

Initial culture of all THP-1 derived cells must be performed in growth medium containing 20% heat-inactivated FBS.

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate TLRs resulting in differentiation of the monocytes and activation of the reporter gene.

- **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin
- **Freezing Medium:** 95% FBS and 5% DMSO

Required Selection 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 should 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 to a larger tube containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS). **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge at 1000-1500 RPM (RCF 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the cells to a T-25 culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
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.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of the cell suspension 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 and are growing well (following at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 10 µg/ml Blasticidin and 100 µg/ml Zeocin™ to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 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 THP1-Dual™ KO-IRF3 cells with less than 20 passages.

REPORTER ASSAY

Cell preparation

1. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 minutes.
2. Remove supernatant and resuspend THP1-Dual™ KO-IRF3 cells at 5 x 10^5 cells/ml in freshly prepared, pre-warmed test medium.

Detection of NF-κB induction

1. Add 20 µl of test compound per well of a flat-bottom 96-well plate. Include a positive control (such as recombinant human TFN-α at 1 ng/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of cell suspension (~100,000 cells) per well.
3. Incubate the plate for 18-24h at 37 °C, 5% CO_2.
4. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
5. Dispense 180 µl of QUANTI-Blue™ Solution per well of a new flat-bottom 96-well plate.
6. Add 20 µl of stimulated THP1-Dual™ KO-IRF3 cell culture supernatant per well.
7. Incubate the plate at 37 °C for 1-3 h.
8. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Detection of IRF induction

Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Add 20 µl of test compound per well of a flat-bottom 96-well plate. Include a positive control (such as recombinant human IFN-β at 1000 IU/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of cell suspension (~100,000 cells) per well.
3. Incubate the plate for 18-24h at 37 °C, 5% CO_2.
4. Prepare QUANTI-Luc™ following the instructions on the enclosed data sheet.
5. 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.
6. Add 10 µl of stimulated THP1-Dual™ KO-IRF3 cell culture supernatant per well into a 96-well white (opaque) or black plate, or a luminometer tube.
7. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.

Note that different IRF responses are observed depending on the type of PRR agonist and transfection reagent used. For more information, please see the cell line validation data sheet.

RELATED PRODUCTS

Product	Description	Cat. Code
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	rep-qlcg1
Blasticidin	Selection antibiotic	ant-bl-1
Normocin™	Antimicrobial agent	ant-nr-1
Zeocin™	Selection antibiotic	ant-zn-1

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