THP1-ASC-GFP Cells

ASC speck reporter monocytes

Catalog code: thp-ascgfp

https://www.invivogen.com/thp1-asc-gfp

For research use only

Version 25B27-AK

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 **THP1-ASC-GFP 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.

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'.

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.

To ensure maximum efficiency, do not passage THP1-ASC-GFP cells more than 20 times. THP1-ASC-GFP cells should be maintained in growth medium supplemented with the selective antibiotic, Zeocin® (100 µg/ml), following every other passage.

Quality Control

- The functionality of THP1-ASC-GFP cells has been tested using inflammasome inducers, such as the microbial toxin Nigericin (NLRP3 inducer), and transfected poly(dA:dT) (AIM2 inducer).
- The stability of this cell line for 20 passages following thawing has been verified.
- THP1-ASC-GFP cells are guaranteed mycoplasma-free.

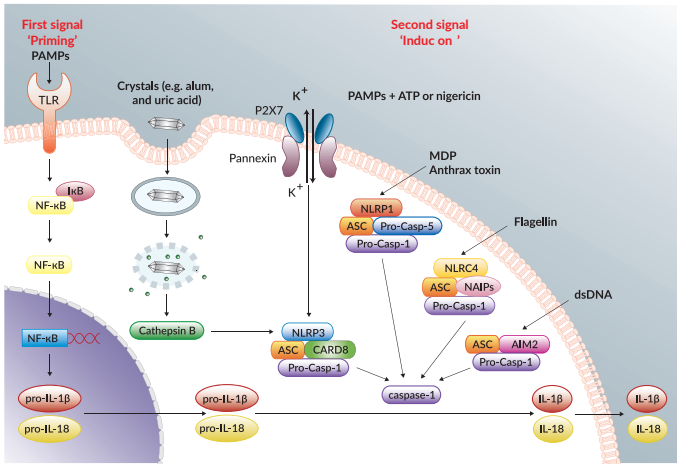
USE RESTRICTIONS

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 to the terms and conditions of all applicable Limited Use Label Licenses.

BACKGROUND

Inflammasomes are multimeric protein complexes that are crucial for host defense against infection and response to endogenous danger signals. Once assembled, they activate caspases, which leads to IL-1β/IL-18 inflammatory cytokine release and pyroptotic cell death. Multiple inflammasomes have been identified, each of which contains one of five distinct sensor proteins: AIM2, NLRC4, NLRP1, NLRP3 or pyrin[1]. These inflammasomes generally also contain the adaptor protein ASC (apoptosis-associated speck-like protein with a CARD; also known as PYCARD) which links the inflammasome sensors to pro-caspase-1[1,2]. NLRP1 and NLCR4 may recruit pro-caspase-1 directly which can lead to pyroptosis but not to IL-1β/IL-18 maturation[1]. In resting cells, ASC is present in a soluble and diffuse cytoplasmic and nuclear form[2]. The assembly of the ASC-dependent inflammasome requires two signals: a first 'priming' signal from a pathogen-associated molecular pattern (PAMP), such as lipopolysaccharide (LPS), followed by a second signal from an inducer such as Nigericin[1]. Activated inflammasome sensors trigger ASC polymerization by prion-like propagation. In most cells, inflammasome activation leads to formation of one large, micrometer-sized, ASC 'speck' per cell[2,3]. Interestingly, extracellular release of ASC specks has been reported *in vitro* and *in vivo*, thus particpating in inflammation propagation[2].



1. Broz P. & Dixit V., 2016. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 16:407-20. 2. Hoss F. *et al.*, 2017. Assembly and regulation of ASC specks. Cell. Mol. Life Sci. 74:1211-29. 3. Stutz A. *et al.*, 2013. ASC speck formation as a readout for inflammasome activation. Methods Mol Biol. 1040:91-101. 4. Zha Q. *et al.*, 2016. ATP-induced inflammasome activation and pyroptosis is regulated by AMP-activated protein kinase in macrophages. Front Immunol. 7:597

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

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

InvivoGen
www.invivogen.com

PRODUCT DESCRIPTION

THP1-ASC-GFP cells are designed to study the activation of ASC-dependent inflammasomes in real time. They are derived from THP-1 human monocytic cells, the most commonly used cellular model for the study of inflammasome activation, as they endogenously express AIM2, ASC, NLRP3 and pro-caspase-1. THP1-ASC-GFP cells stably express an 48.9 kDa ASC::GFP fusion protein that enables the visual monitoring of ASC specks. ASC::GFP expression is driven by an IFN-β minimal promoter fused to five NF-κB binding elements. Green fluorescent protein (GFP) is fused via a six-amino-acid linker at the C terminus of the human ASC protein to avoid interfering with ASC functionality. Hence, in resting cells, no GFP signal is detected. Upon the first step of inflammasome activation ('priming"), NF-kB-dependent ASC::GFP expression is induced and can be observed throughout the cytoplasm. Following the second step of inflammasome activation, ASC::GFP polymerizes to form a macromolecular, micrometer-sized complex. The number of ASC::GFP positive cells and localization of fluorescent ASC specks can be determined by using time-lapse confocal or high-resolution fluorescence microscopy. THP1-ASC-GFP cells are resistant to Zeocin®.

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 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).

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 activation of the reporter gene.

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

Required Selective Antibiotic

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 vial containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS).
4. Centrifuge vial at 150 x g (RCF) for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS). **Do not add selection antibiotics until the cells have been passaged twice.**
6. Transfer the vial contents to a 25 cm^2 tissue 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 freshly prepared freezing medium.
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

- After cells have recovered (after at least two passages), subculture the cells in growth medium (with 10% heat-inactivated FBS). To maintain selection pressure, add 100 µg/ml of Zeocin® to the growth medium every other passage.
- Pass the cells every 3-4 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:

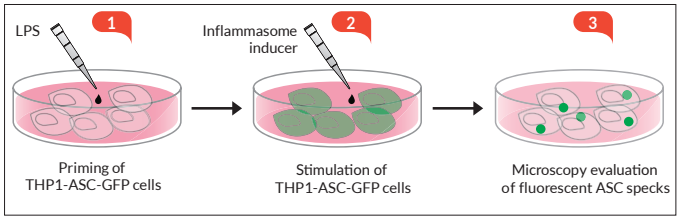
- ***Use THP1-ASC-GFP cells with less than 20 passages.***
- *Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO_2.*

Activation of THP1-ASC-GFP Cells

It is recommended to perform the assay with test medium which does not contain Normocin™ nor Zeocin®.

1. Resuspend THP1-ASC-GFP cells at 2 x 10^6 cells/ml and add 180 µl of this cell suspension per well of a 96-well plate (~ 3.6 x 10^5 cells/well).
2. Prime THP1-ASC-GFP cells with LPS, such as LPS-EK at 1 µg/ml.
3. Incubate for 3 hours at 37 °C in 5% CO_2.
4. Gently remove medium and add 180 µl of test medium.
5. Add 20 µl of an inflammasome inducer, such as Nigericin at 1-5 µM final concentration.
6. Incubate for 1-24 hours at 37 °C in 5% CO_2 and monitor fluorescent ASC specks in real-time using time-lapse confocal or high-resolution fluorescence microscopy using normal FITC filter sets.

Note: Incubation time required to visualize ASC specks depends on the type and concentration of inflammasome inducer.



Spectral properties of GFP

Excitation λ max: 480 nm
Emission λ max: 505 nm

RELATED PRODUCTS

Product	Description	Catalog Code
CPPD Crystals	Inflammasome inducer	tlrl-cppd
LPS-EK (*E.coli* K12)	TLR4 agonist	tlrl-eklps
MSU Crystals	Inflammasome inducer	tlrl-msu
Nigericin	Inflammasome inducer	tlrl-nig
Normocin™	Antimicrobial agent	ant-nr-1
Poly(dA:dT)/LyoVec™	Inflammasome inducer	tlrl-patc
Zeocin®	Selection antibiotic	ant-zn-1

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

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