E. coli OMVs InvivoFit™

Escherichia coli outer membrane vesicles - Non-canonical inflammasome inducer

Catalog code: tlrl-omv

https://www.invivogen.com/ecoli-omvs

For research use only

Version 19B20-ED

PRODUCT INFORMATION

Contents

- 500 µg *E. coli* OMVs InvivoFit™ (quantity of protein measured by BCA) prepared from *Escherichia coli* BL21 and provided lyophilized
- 1.5 mL of sterile endotoxin-free water

Storage and stability

- *E. coli* OMVs InvivoFit™ are provided as a lyophilized powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Store resuspended product at 4 °C or -20 °C. Resuspended product is stable for at least 1 month when properly stored.
- Avoid repeated freeze-thaw cycles.

Quality control

- *E. coli* OMVs InvivoFit™ are guaranteed sterile.
- Endotoxin levels: $>10^5$ EU/mg of OMVs.
- Size range: 35-60 nm
- Activation of TLR2 and TLR4 by *E. coli* OMVs InvivoFit™ has been confirmed using HEK-Blue™ TLR cellular assays.
- Inflammasome activation by *E. coli* OMVs InvivoFit™ has been determined by the induction of pyroptosis in THP1-HMGB1::Lucia™ cells. This is characterized by the release of cytoplasmic contents including the alarmin HMGBI upon membrane rupture. Furthermore, the caspase 11-4/5 inflammasome activates the canonical inflammasome pathway and results in the release of pro-inflammatory cytokines such as IL-1β and IL-18.

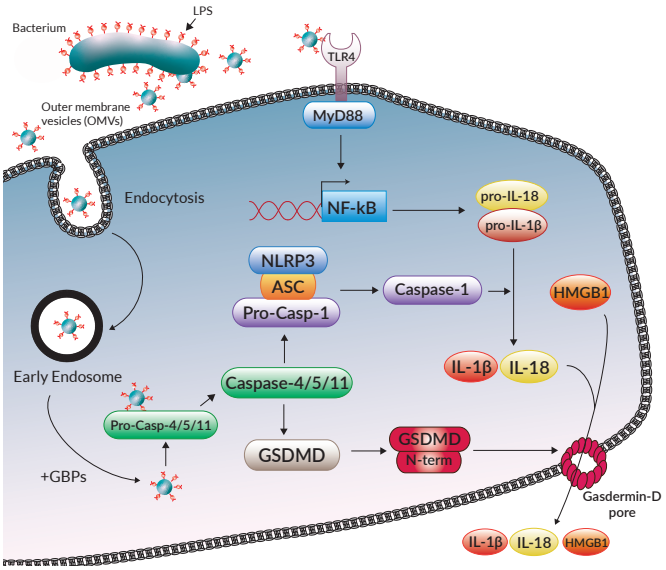
PRODUCT DESCRIPTION

E. coli OMVs background

Pathogenic and commensal Gram-negative bacteria such as *Escherichia coli* secrete small (20-250 nm diameter), non-replicative, immunogenic spherical bodies called outer membrane vesicles (OMVs)[1]. They contain many pathogen-associated molecular patterns (PAMPs) from the parent bacterium such as DNA, RNA, peptidoglycan, lipoproteins, toxins, and lipopolysaccharides (LPS). These PAMPs intiate a range of innate immune signaling pathways by binding to host pattern recognition receptors (PRRs) on the cell surface such as Toll-like receptors (TLRs). Notably, TLR2 recognizes lipoproteins exposed on the surface of OMVs and TLR4 is activated by one of the most abundant constituents of OMVs, LPS (also known as endotoxin).

Role of OMVs in the activation of the innate immune response

Along with interacting with cell surface receptors, OMVs act as a vehicle in the delivery of highly immunogenic molecules across the host cell membrane and into the cytosol, where they can initiate a plethora of immune responses[2]. Once an OMV is endocytosed, it is able to interact with a number of different intracellular host receptors[3]. In particular, LPS, is recognized by and activates murine caspase-11, and the human homologs caspase 4/5, forming a non-canonical inflammasome[4]. The formation of this complex initiates an innate immune response that ultimately leads to pyroptotic cell death through the action of gasdermin D (GSDMD)[5].



1. **Kulp, A. & Kuehn, M.J., 2010.** Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 64, 163-184. 2. **Kaparakis-Liaskos, M. & Ferrero, R.L., 2015.** Immune modulation by bacterial outer membrane vesicles. Nat Rev Immunol 15, 375-387. 3. **Santos, J.C. *et al.*, 2018.** LPS targets host guanylate-binding proteins to the bacterial outer membrane for non-canonical inflammasome activation. EMBO J 37. 4. **Vanaja, S.K. *et al.*, 2016.** Bacterial Outer Membrane Vesicles Mediate Cytosolic Localization of LPS and Caspase-11 Activation. Cell 165, 1106-1119. 5. **Yi, Y.S., 2017.** Caspase-11 non-canonical inflammasome: a critical sensor of intracellular lipopolysaccharide in macrophage-mediated inflammatory responses. Immunology 152, 207-217.

Production and purification of *E. coli* OMVs InvivoFit™

E. coli OMVs InvivoFit™ are purified from late-stationary phase cultures of the commonly used unflagellated *E. coli* BL21 strain grown in a synthetic medium. *E. coli* OMVs InvivoFit™ have then been purified through a series of centrifugation, filtration, and concentration steps. Furthermore, they have been physically characterized using light scattering to ensure a uniformly-sized population of approximately 40-60 nm. Importantly, compared to LPS, OMVs are much more efficient inducers of the caspase 11-4/5 inflammasome both *in vitro* and *in vivo*.

TECHNICAL SUPPORT
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METHODS

Below are the steps for preparing the lyophilized product for use, recommended working concentration ranges for both *in vitro* and *in vivo* use, as well as validated protocols for *in vitro* studies of the activation of the caspase 11-4/5 inflammasome

Preparation of stock suspension (2 mg/ml)

- Add 250 µl of sterile endotoxin free water to the lyophilized *E. coli* OMVs InvivoFit™, and pipette rigorously to ensure complete resuspension.
- Aliquot into working stocks and we recommend to store at -20 °C.

Working concentration ranges

- 0.2 - 100 µg/mL (*in vitro*) and 10 - 100 µg/mouse (*in vivo*).

Activation of the caspase 11-4/5 inflammasome by *E. coli* OMVs InvivoFit™ using THP1-HMGB1::Lucia™ cells

Below is a protocol for determining the effect of *E. coli* OMVs InvivoFit™ on caspase 11-4/5 inflammasome-induced pyroptosis, by detecting the Lucia luciferase activity of HMGB1::Lucia released from THP1-HMGB1::Lucia™ cells.

Note: For the full description of the THP1-HMGB1::Lucia™ cells please visit https://www.invivogen.com/ thp1-hmgb1-lucia

Note: This protocol is for end-point readings of Lucia luciferase activity using a luminometer and can be adapted for use with kinetic measurements.

1. Add 20 µl of *E. coli* OMVs InvivoFit™ per well of a 96-well plate.
2. Add 20 µl of a positive inflammasome inducing control, such as Nigericin (10 µM) in a control well.
3. Prepare a THP1-HMGB1::Lucia™ cell suspension at ~1.1 x 10^6 cells/ml.
4. Dispense 180 µl of cell suspension (~200,000 cells) per well.
5. Incubate at 37 °C in 5% CO_2. Analyze the HMGB1::Lucia released after various incubation times (6-24 hours) using QUANTI-Luc™, a lucia luciferase detection reagent.
6. Prepare the QUANTI-Luc™ assay solution, following the instructions on the data sheet.
7. Transfer 10 µl of THP1-HMGB1::Lucia™ stimulated cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
8. Add 50 µl of QUANTI-Luc™.
9. Proceed immediately with the measurement.

In vitro induction of the caspase 11-4/5 inflammasome in bone marrow-derived macrophages (BMDMs)

Below is a protocol for studying the induction of the caspase 11-4/5 inflammasome in BMDMs by *E. coli* OMVs InvivoFit™

1. Harvest and prepare BMDMs as per your standard protocols.
2. Seed 1 x 10^6 cells per well of a 96-well plate.
3. Add 0.5 - 10 µg of *E. coli* OMVs InvivoFit™ (diluted in PBS) per 250 000 cells
4. Incubate at 37 °C in 5% CO_2 for 6-24 h.
5. Proceed to downstream analysis of caspase 11-4/5 inflammasome induced cell death by measuring the release of pro-inflammatory cytokines such as IL-1β.

Detection of IL-1β using HEK-Blue™ IL-1β cells

Below is the protocol for using InvivoGen's HEK-Blue™ IL-1β cells, which express a NF-κB /AP-1-inducible SEAP reporter, to readily measure the level of IL-1β in the supernatent of your culture. After induction of the caspase 11-4/5 inflammasome by *E. coli* OMVs InvivoFit™, the detection of IL-1β in the supernatant of your culture triggers a signaling cascade leading to the activation of NF-κB and AP-1, and the subsequent production of SEAP.

Note: For the full description of the HEK-Blue™ IL-1β cells please visit https://www.invivogen.com/hek-blue-il1b

Day 1

1. Prepare a HEK-Blue™ IL-1β cell suspension (~330,000 cells/ml).
2. Add 50 µl of activated cell supernatant per well of a flat-bottom 96-well plate.
3. In separate wells, add 50 µl of recombinant human IL-1β (0.25 µg/ml), as the positive control, and 50 µl of recombinant human TNF-α (0.25 µg/ml), as the negative control.
4- Add 150 µl of HEK-Blue™ IL-1β cell suspension (~50 000/ well).
5- Incubate overnight at 37 °C in 5% CO_2.

Day 2

1. Prepare the QUANTI-Blue™ Solution, a SEAP detetion reagent, following the instructions on the data sheet.
2. Add 20 µl of induced HEK-Blue™ IL-1β cell supernatant per well of a flat-bottom 96-well plate.
3. Add 180 µl of resuspended QUANTI-Blue™ Solution per well.
4. Incubate the plate at 37°C incubator for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
THP1-HMGB1-Lucia™ cells	Pyroptosis reporter cell line	thp-gb1lc
HEK-Blue™ IL-1β cells	IL-1β reporter cell line	hkb-il1b
Nigericin	Inflammasome inducer	tlrl-nig
Rec hIL-1β	Recombinant human cytokine	rcyec-hil1b
Rec hTNF-α	Recombinant human cytokine	rcyc-htnfa
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1