293/mTLR4-MD2-CD14 Cells

HEK 293 cells stably transfected with the murine TLR4, MD2 and CD14 genes

Catalog # 293/mtlr4md2cd14

For research use only

Version # 15B09-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293/mTLR4-MD2-CD14 Cells (3-7 x 10^6 cells) in Freezing Medium

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

• 100 µl Blasticidin selective antibiotic (10 mg/ml). Store at -20 °C. Product is stable for 1 year when stored at -20 °C.

• 100 µl Hygromycin B Gold (ultrapure hygromycin B; 100 mg/ml). Store Hygromycin B Gold at 4 °C for 6 months or at -20 °C for 1 year.

• 1 ml Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C. Product is stable for 18 months when stored at -20 °C.

Handling Cells Upon Arrival

Cells must be thawed **immediately** upon receipt and grown according to handling procedures to ensure the best cell viability and assay performance. If you are unable to thaw the cells immediately, frozen cells may be placed in liquid nitrogen until you are ready to thaw and propagate them, however, this may reduce cell viability.

Product Warranty

InvivoGen warrants that cells shall be viable upon shipment from InvivoGen for a period of thirty days, provided they have been properly stored and handled during this period.

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.

293/mTLR4-MD2-CD14 cells should not be passaged more than 20 times to remain fully efficient. 293/mTLR4-MD2-CD14 cells should be maintained in Growth Medium as described below in the presence of Normocin™ (100 µg/ml) and the selective antibiotics, Blasticidin (10 µg/ml) and 50 µg/ml of Hygromycin B Gold. Antibiotic pressure with Blasticidin is required to maintain the plasmid coding for mTLR4 and Hygromycin B Gold to maintain the plasmid coding for MD2 and CD14.

Quality control

Expression of mTLR4, MD2 and CD14 gene was confirmed by RT-PCR. These cells were stimulated with various TLR4 Ligands. These cells are guaranteed mycoplasma-free.

INTRODUCTION

TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS[1]. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionally interact with LPS[2].

Furthermore, a third protein, called CD14, was shown to participate in LPS signaling, leading to NF-κB translocation. This signaling is mediated through several adaptor proteins: MyD88 TIRAP/Mal[3], TRIF/TICAM1 and TRAM/TICAM2[4].

1. Poltorak A. et al., 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science, 282(5396):2085-8. **2. Shimazu R. et al., 1999.** MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med, 189(11):1777-82. **3. Horng T. GM. Barton, and R. Medzhitov, 2001.** TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol, 2(9):835-41. **4. Fitzgerald KA. et al., 2003.** LPS-TLR4 Signaling to IRF-3/7 and NF-{kappa}B Involves the Toll Adapters TRAM and TRIF. J Exp Med. 198(7):1043-1055.

PRODUCT DESCRIPTION

293/mTLR4-MD2-CD14 cells are designed for studying the stimulation of murine TLR4 (mTLR4). 293/mTLR4-MD2-CD14 cells were obtained by co-transfection of the mTLR4, MD2 and CD14 genes. HEK293 cells express endogenous levels of the human genes for TLR1, TLR3, TLR5, TLR6 and NOD1.

Note: The control cell line for 293/mTLR4-MD2-CD14 cells is 293/null cells (cells which do not express TLR4).

USE RESTRICTIONS

These cells are distributed for research purposes only.

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SAFETY CONSIDERATIONS

Biosafety Level:2

293/mTLR4A-MD2-CD14 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to CDC guidelines. The biosafety level may vary depending on the country.

HANDLING PROCEDURES

Required Cell Culture Medium

• Growth Medium: DMEM, 4.5 g/l glucose, 10% (v/v) fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

• Freezing Medium: DMEM, 4.5 g/l glucose, 20% (v/v) fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine, 10% (v/v) DMSO

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% (v/v) 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 vial at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
5- Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of Growth Medium without selective antibiotics.
6- Transfer the vial contents to a 25 cm^2 tissue 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 Growth Medium.
Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
2- Aliquot 1 ml cells into cryogenic vials.
3- Place vials in a freezing container (Nalgene) 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- Maintain and subculture the cells in Growth Medium supplemented with 10 µg/ml Blasticidin and 50 µg/ml of Hygromycin B Gold.
2- Renew Growth Medium 2 times a week.
3- Cells should be passaged when a 70-80% confluency is reached, detach the cells in the presence of PBS by tapping the flask or by using a cell scraper. Do not let the cells grow to 100% confluency.
Note: The response of 293/mTLR4-MD2-CD14 cells can be altered by the action of trypsin. Do not use trypsin to detach 293/mTLR4-MD2-CD14 cells.

TLR4 Stimulation

TLR4 stimulation can be assessed by determining the levels of IL-8 using an ELISA kit or by measuring the activation of NF-κB. InvivoGen has developed a simple and convenient method to evaluate TLR stimulation through NF-κB activation based on the use of an NF-κB-inducible SEAP reporter system (pNiFty-SEAP) and QUANTI-Blue™, a SEAP detection medium.

Day 1: Transfection of 293/mTLR4-MD2-CD14 cells with pNiFty-SEAP

1- Prepare pNiFty-SEAP/LyoVec™ complexes following the instructions provided in the technical data sheet of LyoVec™.
Note: If using another transfection reagent, perform transfection according to the manufacturer's recommendations.
2- Seed 50,000 cells per well of a flat-bottom 96-well plate in 200 µl Growth Medium.
3- Add 10 µl of pNiFty(2)-SEAP/LyoVec™ complexes per well.
4- Incubate the plate at 37°C in a CO2 incubator for 20-24 h.

Day 2: TLR4 Stimulation

- Remove medium and replace with 180 µl of fresh Growth Medium which contains 10% (v/v) heat-inactivated FBS.
Note: Some fetal bovine serum (FBS) may contain alkaline phosphatases that can interfere with SEAP quantification. To ensure that these thermosensitive enzymes are inactive, use heat-inactivated FBS (30 min at 56°C). Heat-inactivated FBS is also commercially available.
- Add 20 µl of each sample per well of a 96-well plate.
- Add 20 µl of a positive control (such as LPS-EK Ultrapure, 100 ng/ml) in one well.
- Add 20 µl of a negative control (such as sterile, endotoxin-free water) in one well.
- Incubate the plate at 37°C in a CO2 incubator for 20-24 h.

Day 3: Detection and Quantification of SEAP

- Prepare QUANTI-Blue™ following the instructions on the pouch.
- Add 180 µl of resuspended QUANTI-Blue™ per well of a 96-well plate.
- Add 20 µl of induced 293/mTLR4-MD2-CD14 Cells supernatant.
- Incubate the plate at 37°C incubator for 1-3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.
Note: For faster reading or high-throughput applications we recommend the use of the one step HEK-Blue™ Detection growth medium. This medium allows for the combined growth of your cells and reading of SEAP activity.

Specificity of 293/mTLR4-MD2-CD14 Cells

As HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1, 293/mTLR4-MD2-CD14 Cells will respond to TLR3, TLR5 and NOD1 ligands. To ensure the specificity of the TLR4 activation, we recommend that you perform experiments with the control cell line 293-null cells. This will avoid misleading results, due to direct activation of NF-κB via a non-TLR4 pathway (e.g. TNF-α activation of NF-κB).

RELATED PRODUCTS

Product	Catalog Code
293-null (Control cell line)	293-null
Blasticidin	ant-bl-1
HEK-Blue™ Detection	hb-det2
Hygromycin B Gold	ant-hg-1
LPS-EK ultrapure (*E. coli K12*)	tlrl-peklps
LyoVec™ (Transfection reagent)	lyec-1
Normocin™	ant-nr-1
MPLAs (synthetic monophosphoryl lipid A)	tlrl-mpls
pNiFty2-SEAP (NF-κB inducible reporter plasmid)	pnifty2-seap
QUANTI-Blue™	rep-qb1