293/hTLR4a Cells

HEK-293 cells stably transfected with human TLR4a gene

Catalog # 293-htlr4a

For research use only

Version # 14H11-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293/hTLR4a 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 (10 mg/ml). Store blasticidin 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/hTLR4a cells should not be passaged more than 20 times to remain fully efficient. 293/hTLR4a cells should be maintained in growth medium supplemented with blasticidin. Antibiotic pressure with blasticidin is required to maintain the plasmid coding for the human TLR4a gene.

Quality control

TLR4 activity is validated upon stimulation with LPS 24 hours after co-transfection of 293/hTLR4a cells with a human CD14/MD-2 expression plasmid and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter plasmid.
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 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.

INTRODUCTION

Toll-like receptor (TLR) 4, the first TLR identified, 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. *et al.*, 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-kappaB involves the toll adapters TRAM and TRIF. J Exp Med. 198(7):1043-55.

PRODUCT DESCRIPTION

293/hTLR4a cells are designed for studying the stimulation of human TLR4 (hTLR4). 293/hTLR4a cells were generated by stable transfection of the HEK293 cell line with the hTLR4a (long isoform, canonical sequence) gene. 293/hTLR4a cells do not express the human MD2 and CD14 genes.

As MD-2 and CD14 are necessary for the LPS-induced responsiveness of TLR4, the co-transfection of 293/hTLR4a cells with the MD-2 and CD-14 is required prior to stimulation.
Notes:
- HEK293 cells express endogeneous levels of TLR1, TLR3, TLR5, TLR6 and NOD1.
- The control cell line for 293/hTLR4a cells is 293/null.

SAFETY CONSIDERATIONS

Biosafety Level: 2

293-hTLR4a 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.

TECHNICAL SUPPORT
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HANDLING PROCEDURES

Required Cell Culture Media

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

• Test Medium: DMEM (4.5 g/l glucose), 10% (v/v) heat-inactivated FBS (30 min at 56ºC), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

Note: Heat-inactivated FBS is also commercially available.

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 (approximately 2 minutes).
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 of the operations 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 T-25 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 3-7 x 10^6 cells/ml in Freezing Medium prepared extemporaneously 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 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.
2. Renew growth medium 2 times a week.
3. Cells should be passaged when a 70-80% confluency is reached. Detach the cells in presence of PBS by tapping the flask or by using a cell scraper. Do not let the cell grow to 100% confluency.

Note: The response of 293/hTLR4a cells can be altered by the use of trypsin. Avoid trypsin to detach 293/hTLR4a cells.

DETECTION OF 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 (secreted embryonic alkaline phosphatase) reporter plasmid (pNiFty-SEAP) and QUANTI-Blue™, a SEAP detection reagent. Alternatively, InvivoGen provides HEK-Blue™ hTLR4 cells (see "Related Products"), a SEAP reporter cell line expressing the hTLR4a, MD-2 and CD-14 genes.

The following protocol describes a method to assess TLR4 stimulation using pNiFty-SEAP, an NF-κB-inducible SEAP reporter plasmid.

Note: TLR4 requires MD-2 and CD14 to signal. 293/hTLR4a cells should be co-transfected with both genes, using the pDUO2-hMD2/CD14 plasmid for example, prior to stimulation.

Day 1: Co-transfection of 293/hTLRa cells with pNiFty-SEAP and pDUO2-hMD2/CD14 plasmids using the transfection reagent LyoVec™.

1. Prepare pNiFty-SEAP / pDUO2-hMD2/CD14 / LyoVec™ complexes by mixing 0.5 µg of each plasmid to 100 µl of LyoVec™ following the instructions provided in the technical datasheet of LyoVec™.

Note: If using another transfection reagent, perform co-transfection according to the manufacturer's recommendations.

2. Seed 50,000 cells of per well of a flat-bottom 96-well plate in 200 µl Growth Medium.
3. Add 10 µl of pNiFty-SEAP / pDUO2-hMD2/CD14 / LyoVec™ complexes per well.
4. Incubate the plate at 37°C in a CO_2 incubator for 20-24h.

Day 2: TLR4 stimulation

1. Remove medium and replace with 180 µl of fresh Test Medium.

Note: FBS may contain alkaline phosphatases that can interfere with SEAP quantification. The Test Medium contains heat-inactivated FBS to eliminate the activity of these thermosensitive enzymes.

2. Add 20 µl of each sample per well of a 96-well plate.
3. Add 20 µl of a positive control (e.g. LPS-EB ultrapure at 100 ng/ml) in one well.
4. Add 20 µl of a negative control (e.g. sterile endotoxin-free water) in one well.
5. Incubate the plate at 37°C in a CO_2 incubator for 20-24h.

Day 3: Detection and Quantification of SEAP

1. Prepare QUANTI-Blue™ following the instructions provided in the technical datasheet.
2. Add 180 µl of QUANTI-Blue™ per well of a 96-well plate.
3. Add 20 µl of induced 293/hTLR4a cells supernatant.
4. Incubate the plate at 37°C in an incubator for 1-3h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Specificity of 293/hTLR4a cells

As their parental cell line, HEK293 cells, express endogenous levels of TLR3, TLR5 and NOD1, 293/hTLR4a cells will respond to TLR3, TLR5 and NOD1 agonists, such as poly(I:C), flagellin and iE-DAP, respectively. In order to identify TLR4-specific responses, we recommend to use 293/null cells (HEK293 cells expressing a blasticidin selectable empty plasmid) as a control cell line.

RELATED PRODUCTS

Product	Catalog Code
293/null (Control cell line)	293-null
Blasticidin (100 mg)	ant-bl-1
FLA-ST Ultrapure (*S. typhimurium* flagellin)	tlrl-epstfla
HEK-Blue™ hTLR4 cells	hkb-htlr4
iE-DAP	tlrl-dap
LPS-EB Ultrapure (*E. coli O111:B4* LPS)	tlrl-3pelps
LyoVec™ (Transfection reagent)	lyec-1
Normocin™ (Antimicrobial agent)	ant-nr-1
pDUO-hMD2/CD14	pduo2-hmd2cd14
pNiFty-SEAP (SEAP reporter plasmid)	pnifty-seap
Poly(I:C)	tlrl-pic
QUANTI-Blue™ (5 pouches)	rep-qb1