293-mTLR1/2 Cells

HEK 293 cells stably transfected with the murine TLR1 and TLR2 genes

Catalog # 293-mtlr12

For research use only

Version # 10J13-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293-mTLR1/2 Cells (5-7 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.
• 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.

PRODUCT DESCRIPTION

293-mTLR1/2 cells are designed for studying the stimulation of murine TLR1/2 (mTLR1/2). 293-mTLR1/2 cells were obtained by co-transfection of the mTLR1 and mTLR2 genes. HEK293 cells express endogenous levels of the human genes for TLR3, TLR5, TLR6 and NOD1. *Note: The appropriate control cell line for 293-mTLR1/2 cells is 293/null cells (cells which do not express TLR2).*

TLR2 is involved in the recognition of a wide array of microbial molecules. TLR2 recognizes peptidoglycan, lipoteichoic acid and lipoprotein from gram-positive bacteria, lipoarabinomannan from mycobacteria, and zymosan from yeast cell wall. TLR2 cooperates with TLR6 in response to diacylated mycoplasmal lipopeptide[1], and associates with TLR1 to recognize triacylated lipopetides[2,3]. Simultaneous expression of the extracellular and intracellular domains of both TLR1 and TLR2 is essential for ligand recognition and subsequent ligand-induced signal activation[4]. Furthermore, pathogen recognition by TLR2 is strongly enhanced by CD14[5]. Stimulation of TLR1/2 triggers a signaling cascade leading to the activation of the transcription factor NF-κB and the production of pro-inflammatory cytokines such as IL-8.

1. Girard R et al., 2003. Lipopolysaccharides from Legionella and Rhizobium stimulate mouse bone marrow granulocytes via Toll-like receptor 2. J Cell Sci. 116:293-302. **2. Ozinsky A. et al., 2000.** The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci USA. 97:13766-71. **3. Thakran S. *et al.*, 2008.** Identification of Francisella tularensis lipoproteins that stimulate the Toll-like receptor (TLR) 2/TLR1 heterodimer. J Biol Chem 283: 3751-9. **4. Sandor F. *et al.*, 2003.** Importance of extra- and intracellular domains of TLR1 and TLR2 in NFkB signaling. J Cell Biol. 162: 1099-10. **5. Lotz S. et al., 2004.** Highly purified lipoteichoic acid activates neutrophil granulocytes and delays their spontaneous apoptosis via CD14 and TLR2. J Leukoc Biol. 75(3):467-77.

SAFETY CONSIDERATIONS

Biosafety Level:2

Handling Cells Upon Arrival

We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability and assay performance. 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-mTLR1/2 cells should not be passaged more than 20 times to remain fully efficient. 293-mTLR1/2 cells should be maintained in Growth Medium as described below in the presence of Normocin™ (100 µg/ml) and the selective antibiotic, Blasticidin (10 µg/ml). Antibiotic pressure with Blasticidin is required to maintain the plasmid coding for mTLR1 and mTLR2.

Quality control

Expression of mTLR1 and mTLR2 genes was confirmed by RT-PCR. These cells were stimulated with various TLR1/2 Ligands. These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

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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.
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 cells grow to 100% confluency.
Note: The response of 293-mTLR1/2 cells can be altered by the action of trypsin. Do not use trypsin to detach 293-mTLR1/2 cells.

TLR1/2 Stimulation
TLR1/2 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-mTLR1/2 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 800,000 cells per well of a 6-well plate in 2 ml Growth Medium.
3- Add 100 µl of pNiFty(2)-SEAP/LyoVec™ complexes
4- Incubate the plate at 37°C in a CO_2 incubator for 20-24 h.

Day 2: TLR1/2 Stimulation
- Add 20 µl of each sample per well of a flat-bottom 96-well plate.
- Add 20 µl of a positive control (such as HKLM, 10^9 cells/ml) in one well.
- Add 20 µl of a negative control (such as sterile, endotoxin-free water) in one well.
- Prepare a cell suspension of 293-mTLR1/2 Cells at ~280,000 cells per ml in Test 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 180 µl of cell suspension (~50,000 cells) per well.
- Incubate the plate at 37°C in a CO_2 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 flat-bottom 96-well plate.
- Add 20 µl of induced 293-mTLR1/2 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-mTLR1/2 Cells
As HEK293 cells express endogenous levels of TLR3, TLR5, TLR6 and NOD1, 293-mTLR1/2 Cells will respond to TLR3, TLR5 and NOD1 ligands. To ensure the specificity of the mTLR1/2 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-TLR1/2 pathway (e.g. TNF-α activation of NF-κB).

RELATED PRODUCTS

Product	Catalog Code
Blasticidin (100 mg)	ant-bl-1
293-null (Control cell line)	293-null
pNiFty2-SEAP (NF-κB inducible reporter plasmid)	pnifty2-seap
LyoVec™ (Transfection reagent)	lyec-1
QUANTI-Blue™ (5 pouches)	rep-qb1
HEK-Blue™ Detection (2 pouches)	hb-det1
Normocin™	ant-nr-1
HKLM (TLR2 ligand)	tlrl-hklm
Pam3CSK4 (TLR1/2 ligand)	tlrl-pms