293-hTLR5-CD14 Cells

HEK 293 cells stably transfected with the human TLR5 and CD14 genes

Catalog # 293-htlr5cd14

For research use only

Version # 10D12-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293-hTLR5-CD14 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.
• 100 µl HygroGold™ (ultrapure hygromycin B; 100 mg/ml). Store HygroGold™ 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.

PRODUCT DESCRIPTION

293-hTLR5-CD14 cells are designed for studying the stimulation of human TLR5 (hTLR5). 293-hTLR5 cells were obtained by co-transfection of the hTLR5 and CD14 genes. HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1. *Note: The control cell line for 293-hTLR5 cells is 293/null cells (do not express CD14 and expression levels of hTLR5 are 100-fold lower than in 293-hTLR5 cells).*

TLR5 is recognizes flagellin from both Gram-positive and Gram-negative bacteria. Stimulation of TLR5 triggers a signaling cascade leading to the activation of the transcription factor NF-κB and the production of proinflammatory cytokines, such as TNF-α, through signaling via the adaptor protein MyD88 and the serine kinase IRAK[1, 2]. TLR5 can generate a proinflammatory signal as a homodimer suggesting that it might be the only TLR participating in flagellin recognition[2]. However, TLR5 may require the presence of a co-receptor or adaptor molecule for efficient ligand recognition and/or signaling[3].

1. Gewirtz AT. *et al*., 2001. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol, 167(4):1882-5. **2. Hayashi F. *et al*., 2001.** The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature, 410(6832):1099-103. **3. Tallant T. *et al*., 2004.** Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-kappa B and proinflammatory gene program activation in intestinal epithelial cells. BMC Microbiol. 4(1):33.

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-hTLR5 cells should not be passaged more than 20 times to remain fully efficient. 293-hTLR5 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) and HygroGold™ (ultrapure hygromycin B; 50 µg/ml). Antibiotic pressure with Blasticidin is required to maintain the plasmid coding for hTLR5 and hygromycin B is required to maintain the plasmid coding for CD14.

Quality control

Expression of TLR5 and CD14 genes was confirmed by RT-PCR. These cells were stimulated with various TLR5 Ligands. 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

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

TECHNICAL SUPPORT
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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 HygroGold™ (ultrapure hygromycin B; 50 µg/ml).
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-hTLR5-CD14 cells can be altered by the action of trypsin. Do not use trypsin to detach 293-hTLR5-CD14 cells.

TLR5 Stimulation

TLR5 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. Alternatively, Invivogen provides HEK-Blue™ hTLR5 Cells (cat. code hkb-htlr5), a SEAP reporter cells line expressing the hTLR5 gene.

Day 1: Transfection of 293-hTLR5-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 CO_2 incubator for 20-24 h.

Day 2: TLR5 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 RecFLA-ST (recombinant flagellin from *S. typhimurium*), 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 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 96-well plate.
- Add 20 µl of induced 293-hTLR5-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-hTLR5-CD14 Cells

As HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1, 293-hTLR5-CD14 cells will respond to TLR3, TLR5 and NOD1 ligands. To ensure the specificity of the hTLR5 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-hTLR5 pathway (e.g. TNF-α activation of NF-κB). In non-saturating conditions, the response to TLR5 ligands in 293-hTLR5-CD14 cells is normally between 10 and 100-fold higher than in 293-null cells.

RELATED PRODUCTS

Product	Catalog Code
Blasticidin (100 mg)	ant-bl-1
HygroGold™ (1 g)	ant-hg-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
FLA-BS (Flagellin from *B.subtilis*; TLR5 ligand)	tlrl-bsfla
FLA-ST (Flagellin from *S. typhimurium*; TLR5 ligand)	tlrl-stfla
FLA-ST Ultrapure (TLR5 ligand)	tlrl-pstfla
RecFLA-ST (recombinant flagellin; TLR5 ligand)	tlrl-flic