293XL-hTLR7

293XL cell line expressing the human TLR7 gene

Catalog # 293xl-htlr7

For research use only

Version # 10D14-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293XL-hTLR7 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

293XL-hTLR7 cells are designed for studying the stimulation of human TLR7 (hTLR7). 293XL-hTLR7 cells were obtained by co-transfection of the hTLR7 and the human antiapoptotic Bcl-XL genes. HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1. *Note: The control cell line for 293XL-hTLR7 cells is 293XL/null cells (which do not express hTLR7).*

TLR7, which is abundantly expressed in lung, placenta, spleen and PBL, is phylogenetically close to TLR8 and TLR9[1]. TLR7 recognizes small synthetic molecules such as loxoribine and R848, an imidazoquinoline compound[2]. TLR7 signaling involves the MyD88-dependent signaling cascade and induces the production of IFN-α, TNF-α and IL-12. Sequence-specific single-stranded RNA (ssRNA) was identified as the natural ligand of TLR7[3, 4]. ssRNA sequences derived from HIV-1 or the influenza virus were shown to induce the production of proinflammatory cytokines in PDC. Furthermore, it has been demonstrated that TLR7 is involved in sequence-specific sensing of ssRNA in human macrophages[5]. TLR7 signaling is abrogated by chloroquine indicating that it is dependent on endosomal acidification.

1. Chuang TH. & RJ. Ulevitch, 2000. Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. Eur Cytokine Netw, 11:372-8. **2. Hemmi H. *et al.*, 2002.** Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol, 3:196-200. **3. Heil F. *et al.*, 2004.** Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 303:1526-9. **4. Diebold SS. *et al.*, 2004.** Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 303:1529-31. **5. Gantier MP. *et al.*, 2008.** TLR7 is involved in sequence-specific sensing of single-stranded RNAs in human macrophages. J immunol. 180; 2117-24.

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.

293XL-hTLR7 cells should not be passaged more than 20 times to remain fully efficient. 293XL-hTLR7 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 hTLR7.

Quality control

Expression of hTLR7 gene was confirmed by RT-PCR. These cells were stimulated with various TLR7 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

SAFETY CONSIDERATIONS

Biosafety Level:2

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

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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.
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 293XL-hTLR7 cells can be altered by the action of trypsin. Do not use trypsin to detach 293XL-hTLR7 cells.

TLR7 Stimulation

TLR7 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™ hTLR7 Cells (cat. code hkb-htlr7), a SEAP reporter cells line expressing the hTLR7 gene.
5- Determine SEAP levels using a spectrophotometer at 620-655 nm.

Day 1: Transfection of 293XL-hTLR7 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: TLR7 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 CL264, 5 µg/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 293XL-hTLR7 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 293XL-hTLR7 Cells

As HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1, 293XL-hTLR7 cells will respond to TLR3, TLR5 and NOD1 ligands. To ensure the specificity of the hTLR7 activation, we recommend that you perform experiments with the control cell line 293XL-null cells. This will avoid misleading results, due to direct activation of NF-κB via a non-hTLR7 pathway (e.g. TNFα activation of NF-κB).

RELATED PRODUCTS

Product	Description	Catalog Code
Blasticidin (100 mg)		ant-bl-1
293XL/null (Control cell line)		293xl-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
CL264 (TLR7 ligand)		tlrl-c264
CL264 Biotin (labeled TLR7 ligand)		tlrl-bc264
CL264 FITC (labeled TLR7 ligand)		tlrl-fc264