293-mTLR6 Cells

HEK 293 cells stably transfected with the murine TLR6 gene

Catalog # 293-mtlr6

For research use only

Version # 10D14-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293-mTLR6 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-mTLR6 cells were obtained by stable transfection of the murine TLR6 (mTLR6) gene. HEK293 cells express endogenous levels of the human genes for TLR1, TLR3, TLR5, TLR6 and NOD1. *Note: 293-mTLR6 cells can be used as control cells for 293-mTLR2/6 cells.*

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-mTLR6 cells should not be passaged more than 20 times to remain fully efficient. 293-mTLR6 cells should be maintained in Growth Medium as described below in the presence of Normocin™ (100 µg/ml) and the selective antibiotic, 10 µg/ml of Blasticidin. Antibiotic pressure with Blasticidin to maintain the plasmid coding for mTL6.

Quality control

Expression of mTLR6 gene was confirmed by RT-PCR. 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 of 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-mTLR6 cells can be altered by the action of trypsin. Do not use trypsin to detach 293-mTLR6 cells.

Specificity of 293-mTLR6 Cells

As HEK293 cells express endogenous levels of the human genes for TLR1, TLR3, TLR5, TLR6 and NOD1, 293-mTLR6 Cells will respond to TLR3, TLR5 and NOD1 ligands.

RELATED PRODUCTS

Product	Catalog Code
Blasticidin (100 mg)	ant-bl-1
Normocin™	ant-nr-1