293-mNOD2 Cells

293 cells expressing the murine NOD2 gene

Catalog # 293-mnod2

For research use only

Version # 10E24-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293-mNOD2 cells (5-7 x 10^6 cells) in Freezing Media
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-mNOD2 cells are designed for studying the stimulation of murine NOD2 (mNOD2). 293-mNOD2 cells were obtained by stable transfection of the mNOD2 gene into HEK293 cells. HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1. *Note: The control cell line for 293-mNOD2 cells is 293-null cells (cells which do not express NOD2).*

NOD2 (CARD15) is a member of the family of Nod-like receptors (NLRs, also known as CATERPILLER), characterized by a nucleotide-oligomerization domain (NOD) and ligand-recognizing leucine-rich repeats. NOD2 is an intracellular pattern-recognition molecules involved in the recognition of peptidoglycan (PGN). NOD2 detects specific motifs within the PGN. NOD2 recognizes the muramyl dipeptide (MDP) structure found in almost all bacteria. It signals via the serine/threonine RIP2 (RICK, CARDIAK) kinase which interacts with IKK leading to the activation of NF-κB and the production of inflammatory cytokines such as TNF-α and IL-6[1]. In addition to the NF-κB pathway, NOD2 stimulation induces the activation of MAPKs[2]. CARD9 has been implicated in the selective control of NOD2-dependent p38 and JNK signaling[3]. The physiological importance of NOD2 in immune responses is evident from the linkage of their mutations with inflammatory diseases in humans. Genetic variation in NOD2 is associated with Crohn's disease, one of the major forms of inflammatory bowel diseases[4].

1. Inohara N. et al., 2000. An induced proximity model for NF–κB activation in the Nod1/RICK and RIP signaling pathways. J. Biol. Chem. 275: 27823-27831. **2. Kobayashi KS. et al., 2005.** Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307: 731-734. **3. Hsu YM. et al., 2007.** The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. Nat Immunol. 8(2):198-205. **4. Ogura Y. et al., 2001.** A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411: 603-606. **5. Hysi P. et al., 2005.** NOD1 variation, immunoglobulin E and asthma. Hum. Mol. Genet. 14: 935-941.

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-mNOD2 Cells should not be passaged more than 20 times to remain fully efficient. 293-mNOD2 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 mNOD2.

Quality control

Expression of the murine NOD2 gene was confirmed by RT-PCR. 293-mNOD2 Cells were stimulated by NOD2 agonists. 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
• Test Medium: DMEM, 4.5 g/l glucose, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (30 min at 56°C)

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 Media 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-mNOD2 Cells can be altered by the action of trypsin. Do not use trypsin to detach 293-mNOD2 Cells.

NOD2Stimulation
NOD2 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-mNOD2 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 CO2 incubator for 20-24 h.

Day 2: NOD2 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 L18-MDP, 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 16-20 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-mNOD2 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-mNOD2 Cells
As HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1, 293-mNOD2 Cells will respond to TLR3, TLR5 and NOD1 ligands. To ensure the specificity of the mNOD2 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 the reporter gene via a non-NOD2 pathway (e.g. TNFα activation of NF-κB).

RELATED PRODUCTS

Product	Catalog Code
Blasticidin (100 mg)	ant-bl-1
QUANTI-Blue™ (5 pouches)	rep-qb1
HEK-Blue™ Detection (2 pouches)	hb-det1
Normocin™	ant-nr-1
N-Glycolyl-MDP (NOD2 ligand)	tlrl-gmdp
L18-MDP (NOD2 ligand)	tlrl-lmdp
MDP (NOD2 ligand; L-D isoform)	tlrl-mdp
MDP Control (Inactive D-D isoform)	tlrl-mdpc
MDP Biotin (labeled NOD2 ligand)	tlrl-bmdp
MDP FITC (labeled NOD2 ligand)	tlrl-fmdp
293 / Null Cells	293-null