

# 293 / null Cells

293 Control cell line

Catalog # 293-null

For research use only

Version # 12112-MM

## PRODUCT INFORMATION

### Contents and Storage

- 1 vial of 293 / null Cells (5-7 x 10<sup>6</sup> cells) in Freezing Medium
- IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.*
- 100 µl Blastidicin 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 / null cells are the control cell line for 293 / TLR and 293/ NOD clones. HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.

### Handling Cells Upon Arrival

Cell must be propagated upon arrival using the provided procedure. DO NOT FREEZE CELLS UPON ARRIVAL. This will ensure the best cell viability and assay performance. The first propagation of cells should be for generating stocks for future use.

### 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 / null cells should not be passaged more than 20 times after you have received them. 293 / null cells should be maintained in Growth Medium in the presence of the selective antibiotic, Blastidicin (10 µg/ml).

### Quality control

- Cells were tested as negative controls in TLR-ligand induction experiments by transient transfection assay with an NF-κB inducible reporter plasmid. Induction was performed with various TLR ligands. The cells were found to not activate NF-κB with induction by all ligands with the exception of TLR3, TLR5 and NOD1 ligands, as HEK293 cells express endogenous levels of TLR3, TLR5 and NOD1.
- 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](mailto: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
- 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<sup>2</sup> tissue culture flask containing 5 ml of Growth Medium without selective antibiotics.
- 7- Place the culture at 37°C in 5% CO<sub>2</sub>.

## TECHNICAL SUPPORT

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### Frozen Stock Preparation

1- Resuspend cells at a density of  $5-7 \times 10^6$  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  $-80^\circ\text{C}$  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  $10 \mu\text{g/ml}$  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 / null cells can be altered by the action of trypsin. Do not use trypsin to detach 293 / null cells.*

### An example of NF- $\kappa$ B Stimulation

InvivoGen has developed a simple and convenient method to evaluate NF- $\kappa$ B activation based on the use of an NF- $\kappa$ B-inducible SEAP reporter system (pNiFty-SEAP) and QUANTI-Blue™, a SEAP detection medium.

### Day 1: Transfection of 293 / null 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  $\mu\text{l}$  Growth Medium.

3- Add 10  $\mu\text{l}$  of pNiFty(2)-SEAP/LyoVec™ complexes per well.

4- Incubate the plate at  $37^\circ\text{C}$  in a  $\text{CO}_2$  incubator for 20-24 h.

### Day 2: NF- $\kappa$ B Stimulation

- Add 20  $\mu\text{l}$  of each sample per well of a flat-bottom 96-well plate.

- Add 20  $\mu\text{l}$  of a positive control (such as TNF- $\alpha$ , 100 ng/ml) in one well.

- Add 20  $\mu\text{l}$  of a negative control (such as sterile, endotoxin-free water) in one well.

- Prepare a cell suspension of 293/ null cells at  $\sim 280,000$  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  $56^\circ\text{C}$ ). Heat-inactivated FBS is also commercially available.*

- Add 180  $\mu\text{l}$  of cell suspension ( $\sim 50,000$  cells) per well.

- Incubate the plate at  $37^\circ\text{C}$  in a  $\text{CO}_2$  incubator for 16-20 h.

### Day 3: Detection and Quantification of SEAP

- Prepare QUANTI-Blue™ following the instructions on the pouch.

- Add 180  $\mu\text{l}$  of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.

- Add 20  $\mu\text{l}$  of induced 293 / null cells supernatant.

- Incubate the plate at  $37^\circ\text{C}$  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 / null cells

As 293 / null cells express endogenous levels of TLR3, TLR5 and NOD1, 293 / null cells will respond to TLR3, TLR5 and NOD1 ligands.

## RELATED PRODUCTS

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

### TECHNICAL SUPPORT

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