293-hMD2-CD14 Cells

HEK 293 cells stably transfected with the human MD2 and CD14 genes

Catalog code: 293-hmd2cd14

https://www.invivogen.com/293-md2-cd14

For research use only

Version 18I26-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of 293-hMD2-CD14 Cells (3-7 x 10^6 cells) in freezing medium
IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.
• 1 ml of Hygromycin B Gold™ (ultra-pure hygromycin B; 100 mg/ml). Store at 4°C or at -20°C.*
• 1 ml of Normocin™ (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C.*
*The expiry date is specified on the product label.

Handling Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.
*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*
Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

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. Cells should not be passaged more than 20 times to remain fully efficient.
293-hMD2-CD14 cells should be maintained in growth medium supplemented with Normocin™ (100 µg/ml) and the selective antibiotic hygromycin B (50 µg/ml). Antibiotic pressure with hygromycin B is required to maintain the plasmid coding for MD2 and CD14.

Quality Control

• Expression of MD2 and CD14 genes has been confirmed by RT-PCR.
• The stability for 20 passages following thawing has been verified.
• 293-hMD2-CD14 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 to 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.

PRODUCT DESCRIPTION

293-hMD2-CD14 cells were obtained by co-transfection of the human MD2 and CD14 genes. HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1.
Note: 293-hMD2-CD14 cells can be used as control cells for 293-hTLR4A-MD2-CD14 cells.

SAFETY CONSIDERATIONS

Biosafety Level 2

293-hMD2-CD14 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to CDC guidelines. The biosafety level may vary depending on the country.

HANDLING PROCEDURES

Required Cell Culture Medium

• Growth Medium: DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) fetal bovine serum, (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™
• Freezing Medium: DMEM, 20% (v/v) FBS, 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.

TECHNICAL SUPPORT
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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 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 50 µg/ml of Hygromycin B Gold™.
2. Renew growth medium twice 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-hMD2-CD14 cells can be altered by the action of trypsin. Do not use trypsin to detach 293-hMD2-CD14 cells.

Specificity of 293-hMD2-CD14 Cells

As HEK293 cells express endogenous levels of TLR1, TLR3, TLR5, TLR6 and NOD1, 293-hMD2-CD14 Cells will respond to TLR3, TLR5 and NOD1 ligands.

RELATED PRODUCTS

Product	Catalog Code
Hygromycin B Gold™	ant-hg-1
Normocin™	ant-nr-1