293-hMyD88 Cells

HEK293 cells with constitutive expression of human (h)MyD88 for SARS-CoV-2 cell fusion assays

Catalog code: 293-hmyd

https://www.invivogen.com/293-hmyd88

For research use only

Version 23C21-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 293-hMyD88 cells in a cryovial or shipping flask

IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

- 1 ml of Puromycin™ (10 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.

Note: Data sheets for all components are available on our website.

Handling Frozen 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.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Cells will undergo genotypic changes over time resulting in reduced responsiveness 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-hMyD88 cells should not be passaged more than 20 times to remain fully functional.

Quality Control

- Transient transfection with pUNO1-Spike and fusion with ACE2-expressing HEK293 cells has been validated.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

CELL LINE DESCRIPTION

293-hMyD88 cells were generated from the human embryonic kidney (HEK)-293 cell line, through the stable and constitutive expression of the human (h)*MyD88* gene. MyD88 is the canonical adaptor in several inflammatory signaling pathways downstream of members of the Toll-like receptor (TLR) and interleukin-1 (IL-1) families. MyD88 links these receptors to IL-1R-associated kinase (IRAK) family kinases, which ultimately leads to the activation of NF-κB-dependent signaling[1].

Constitutive activation of MyD88 in 293-hMyD88 cells is achieved through the introduction of the gain-of-function L256P mutation. Notably, this mutant MyD88 is known to spontaneously assemble and lead to persistent NF-κB activation within the cell[1]. These cells are resistant to Puromycin.

1. Deguine J. & Barton G.M. 2014. MyD88: a central player in innate immune signaling. F1000Prime Reports, 6:97, doi:10.12703/P6-97.

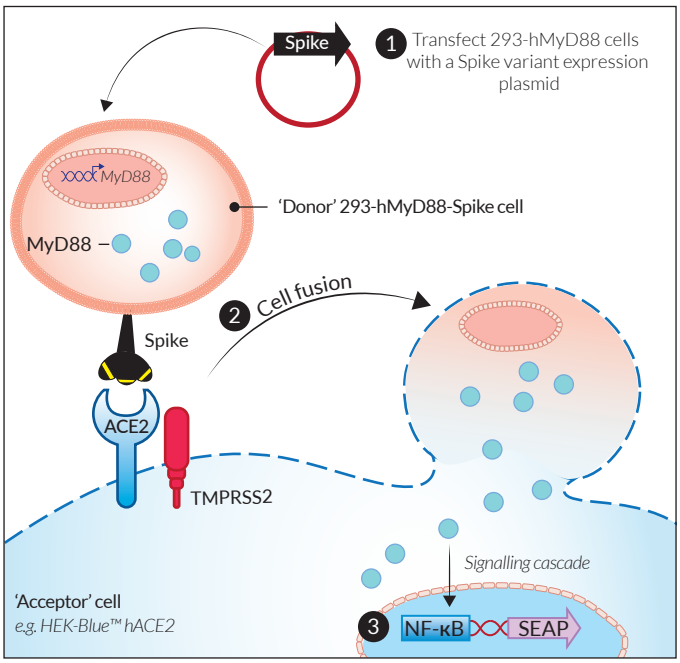
APPLICATION

293-hMyD88 cells have been specifically designed for use in InvivoGen's assay to study SARS-CoV-2 Spike-ACE2-dependent cell fusion. The assay relies on the transfer of MyD88 from the 'donor cell line' to an 'acceptor cell line' expressing an inducible NF-κB-SEAP reporter gene.

- **'Donor cell line'** - transient or stable transfection of 293-hMyD88 with an optimized expression plasmid featuring one of the Spike variants (e.g. UK B.1.1.7 or South Africa B.1.351).
- **'Acceptor cell line'** - InvivoGen has engineered HEK293- and A549-derived NF-κB-SEAP reporter cells, HEK-Blue™ and A549-Dual™, to stably express the SARS-CoV-2 receptors, ACE2 and TMPRSS2.

Upon co-culture of these cell lines, Spike will bind to ACE2 and be proteolytically cleaved by TMPRSS2, and trigger cell fusion. The overexpressed MyD88 in the 'donor' cell will activate a signalling cascade in the 'acceptor' cell that leads to NF-κB-dependent SEAP production. SEAP is readily assessed in the co-culture supernatant using the SEAP detection reagent, QUANTI-Blue™ Solution.

Note: For more information visit https://www.invivogen.com/cell-fusion



USER 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

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FAQ Any questions about our cell lines? Visit our FAQ page.

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SAFETY CONSIDERATIONS

Biosafety Level 2

293-hMyD88 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these thermosensitive enzymes.

- **Required Selection Antibiotics:** Puromycin™

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 (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All of the steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge tube at 200-300 x *g* for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium.
6. Transfer the contents to a T-25 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 5-7x 10^6 cells/ml in freshly prepared freezing medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of cell suspension 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 1 µg/ml of Puromycin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Cell Handling Recommendations

To ensure the best results, use 293-hMyD88 cells with less than 20 passages.

CELL FUSION ASSAY

Generation of "donor cells" using 293-hMyD88 cells

1. Wash cells with PBS and detach cells with trypsin.
2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and resuspend cells at 0.3 x 10^6 cells/ml in fresh, pre-warmed growth medium
4. Add 3ml of cell suspension (~1.0 x 10^6 cells) per well of a 6-well plate.
5. Prepare LyoVec™ complex: Combine 1.5 µg pUNO1-Spike with 150 µL LyoVec™ and incubate at room temperature for 30 mins.

Note: InvivoGen offers a comprehensive collection of expression plasmids encoding various Spike variants (e.g. UK, South Africa etc.).

Note: For more information: https://www.invivogen.com/sars2-spike-vectors

6. Add 150 µl of prepared complex to the cell-containing wells.
7. Incubate the plate for 24h or 48h at 37°C, 5% CO_2.

Co-culture of 'donor' and 'acceptor' cells

1. Wash pre-prepared transfected cells (293-hMyD88-Spike) with PBS and detach in PBS by tapping the plate.
2. Centrifuge cells at 300 x g (RCF) for 5 min.
3. Remove supernatant and prepare a suspension at 1.0 x 10^6 cells/ml in fresh, pre-warmed growth medium.
4. Prepare a 1:2 serial dilution of the 293-hMyD88-Spike cells in a 96-well plate, starting with a final concentration of 1.0 x 10^5 cells/well. Final volume of 100 µl per well.
5. Prepare "acceptor cells" following the instructions on the specific product data sheet. Briefly, harvest the cells and prepare a cell suspension at 2 x10^5 cell/ml in fresh pre-warmed growth medium.

*Note: For the **HEK-Blue™ hACE2** product datasheet visit: https://www.invivogen.com/hek-blue-hace2-cells*

*Note: For the **A549-Dual™ hACE2-TMPRSS2** product data sheet visit: https://www.invivogen.com/a549dual-hace2tmprss2-cells*

6. Add 100 µl of the "acceptor cells" suspension (20,000 cells) per well.
7. Incubate the plate for 24h at 37°C, 5% CO_2.

Measuring cell fusion

1. Prepare QUANTI-Blue™ Solution following the instructions on the product data sheet.
2. Dispense 180 µl of QUANTI-Blue™ Solution per well of a new flat-bottom 96-well plate.
3. Add 20 µl of cell fusion supernatant per well.
4. Incubate the plate at 37°C for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Assessing inhibitors of cell fusion

This protocol can be adapted to study the effect of various inhibitors, such as small molecules or antibodies, on Spike-ACE2-dependent cell fusion. The exact conditions will need to be optimized (e.g. donor:acceptor cell ratio, inihibitor concentration etc.).

RELATED PRODUCTS

Product	Cat. Code
Puromycin	ant-pr-1
Normocin™	ant-nr-1
HEK-Blue™ hACE2 Cells	hkb-hace2
A549-Dual™ ACE2-TMPRSS2 Cells	a549-hace2tpsa
pUNO1-SpikeV1 (G614 variant)	p1-spike-v1
pUNO1-SpikeV2 (UK variant)	p1-spike-v2
pUNO1-SpikeV3 (South Africa variant)	p1-spike-v3
QUANTI-Blue™ Solution	rep-qbs

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