293-SARS2-S Cells

HEK293 cells overexpressing the SARS-CoV-2 Spike (S) protein

Catalog code: 293-cov2-s

https://www.invivogen.com/293-sars2-spike

For research use only

Version 21E05-ED

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 293-SARS2-S 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 Blasticidin™** (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-SARS2-S cells should not be passaged more than 20 times to remain fully functional.

Quality Control

- The overexpression of the SARS-CoV-2 Spike gene has been verified by RT-qPCR
- Upon transient tranfection with the pUNO1-hMyD88 plasmid, 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.

BACKGROUND

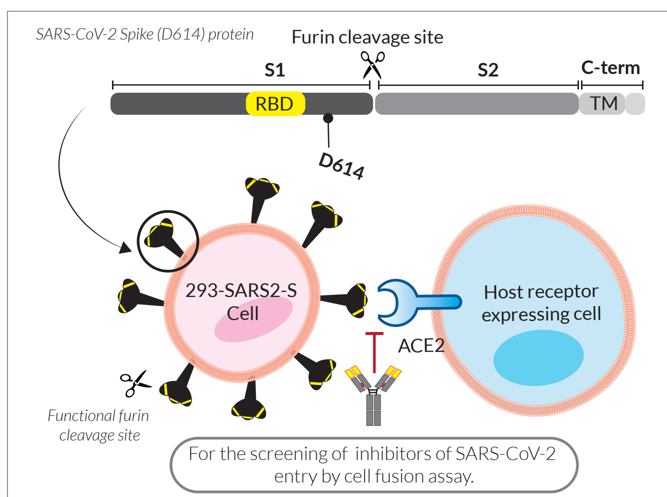
Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates membrane fusion and viral entry into target cells upon binding to the host receptor ACE2 and the proteolytic activity of TMPRSS2[1]. The S protein consists of an N-terminal ectodomain, a transmembrane anchor, and a short C-terminal cytoplasmic tail. The ectodomain contains the S1 subunit, which encodes the receptor binding domain (RBD), a key target in treatment and vaccination strategies against COVID-19, as well as the S2 subunit, needed for membrane fusion[2]. Notably, a furin cleavage sequence (RRxR) is found within a polybasic cleavage site (681-PRRSR/SVA-688) at the boundary between the S1 and S2 domains. It is suggested that furin pre-primes the S protein during its production[3].

CELL LINE DESCRIPTION

293-SARS2-S cells were generated from the human embryonic kidney (HEK)-293 cell line, by stable transfection of the original Wuhan-Hu-1 SARS-CoV-2 Spike (D614) gene with a functional furin cleavage site. As reported in the literature to improve expression of the Spike protein in mammalian cells, the last 19 amino acids, which contain the ER-retention motif, have been removed[4]. These cells are resistant to Blasticidin.

APPLICATION

293-SARS2-S cells express the original Wuhan-Hu-1 Spike protein and have been specifically designed to study Spike-ACE2-dependent cell fusion. InvivoGen has developed a simple reporter assay utilizing 293-SARS-S cells and our SARS-CoV-2 permissive reporter cells such as HEK-Blue™ hACE2 and A549-Dual™ hACE2-TMPRSS2 cells (*for detailed protocol see otherside*). This assay is ideal for screening small molecule inhibitors and/or antibodies that target either the Spike or the host receptors.



1. Hoffmann M. *et al.* 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. 2. Walls A.C. *et al.* 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281- 292.e6. 3. Shang J. *et al.* 2020. Cell entry mechanisms of SARS-CoV-2. PNAS. 1117 (21) 11727-11734. 4. Ou, X. et al. 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11, 1620.

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

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

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

SAFETY CONSIDERATIONS

Biosafety Level 2

293-SARS2-S 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:** Blasticidin

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 without selective antibiotics.
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 10 µg/ml of Blasticidin
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.

Note: The surface expression of Spike will be altered by the action of trypsin. We recommend you add pre-warmed phosphate buffered saline (PBS) and detach cells by tapping the flask.

Cell Handling Recommendations

To ensure the best results, use 293-SARS2-S cells with less than 20 passages.

CELL FUSION ASSAY

InvivoGen has developed a protocol for studying cell fusion using the 293-SARS2-S cells and SARS-CoV-2 permissive cell lines such as HEK-Blue™ hACE2 or A549-Dual™ hACE2-TMPRSS2.

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

Generation of "donor cells" using 293-SARS2-S cells

1. Wash cells with PBS and detach cells in PBS by tapping the flask.
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-hMyD88 with 150 µL LyoVec™ and incubate at room temperature for 30 mins.
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-SARS2-S-hMyD88) 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-SARS2-S-hMyD88 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
Blasticidin	ant-bl-1
HEK-Blue™ hACE2 Cells	hkb-hace2
pUNO1-hMyD88	puno1-hmyd88