293-SARS2-S-dfur Cells

HEK293 cells overexpressing the SARS-CoV-2 Spike (S) protein with an inactivated furin site

Catalog code: 293-cov2-sdf

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

For research use only

Version 21C28-ED

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 293-SARS2-S-dfur 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-dfur 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 flow cytometry.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

BACKGROUND

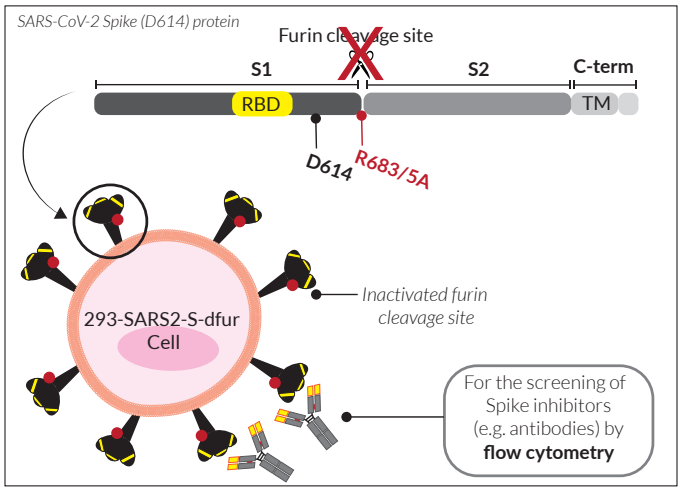
Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates membrane fusion and viral entry into target cells upon binding to the host receptor ACE2 and the proteolytic activity of TMPRSS2[1]. The S protein consists of an N-terminal ectodomain, a transmembrane anchor, and 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 furin pre-primes the SARS-CoV-2 S protein during its production[3].

CELL LINE DESCRIPTION

293-SARS2-S-dfur cells were generated from the human embryonic kidney (HEK)-293 cell line, by transfection of the original Wuhan-Hu-1 SARS-CoV-2 Spike (D614) gene using InvivoGen's pUNO1-Spike-dfur plasmid. The spike furin cleavage site has been inactivated (dfur) through the replacement of arginine with alanines at position 683 and 685. This faciliates improved surface expression and detection for flow cytometry. As reported in the literature to improve expression of the Spike protein, 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-dfur are characterized by high surface expression of the full intact SARS-CoV-2 Spike protein. Therefore, they are ideal for using flow cytometry to screen for Spike-targeting antibodies in sera from infected and/or vaccinated individuals.



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

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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-SARS2-S-dfur 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-dfur cells with less than 20 passages.

RELATED PRODUCTS

Product	Cat. Code
Blasticidin	ant-bl-1
Normocin™	ant-nr-1

COVID-19 Product Range

HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
Anti-CoV2RBD-c1-hIgG1	Recombinant Antibody	cov2rbdc1-mab1
Anti-CoV2RBD-c2-hIgG1	Recombinant Antibody	cov2rbdc2-mab1

For a complete list of InvivoGen's COVID-19 related products visit: https://www.invivogen.com/covid-19