

# **Human ACE2 Expression in HEK293T Cell Line Product Manual**

Catalog Number	Product name	Amount
SC076	HEK293T / human ACE2 Expression Cell Line	1.0 ml / vial
		(2-5 million cells)

Storage: Liquid Nitrogen.

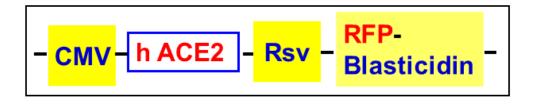
#### **Product Description**

HEK293T cell is a human cell line, derived from the HEK 293 cell line, that expresses a mutant version of the SV40 large T antigen. It is commonly used in biology for protein expression and production of recombinant retroviruses. Due to the expression of SV40 large T antigen, transfected plasmid DNAs that carry the SV40 origin of replication can replicate in 293T and will transiently maintain a high copy number; this can greatly increase the amount of recombinant protein.

ACE2 (angiotensin I converting enzyme 2) is cell surface receptor, mainly expressed in vascular endothelial cells. It acts as a entry point into human cells for some coronaviruses, including the SARS virus and COVID-19 (SARS-CoV-2).

This ACE2 over-expression stable cell line in HEK293T is transformed via lentiviral system. It constitutively expresses high level of human ACE2 gene, and can be used for in vitro screening and characterization of antibodies, vaccines, or drug candidates against SARS-CoV-2, COVID-19 coronavirus.

The human ACE2 coding sequence (100% identical to CD region of NCBI accession ID <u>NM 021804</u>) is expressed under a strong promoter (an enhanced CMV promoter). It demonstrated a high binding affinity to anti-hACE2 antibody. This cell line carries the **RFP-Blasticidin** Dual selection marker under the RSV promoter. The following expression cassette was verified in cell line's genome.



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### **Culture procedures**

- 1. Thaw the frozen vial of cells quickly in a 37°C water bath (1-3 min), decontaminate the outside of the vial with 75% ethanol.
- 2. Transfer the entire contents of the cryovial into a T-75 cm<sup>2</sup> flask containing 15 ml of prewarmed complete medium. Incubate the cells overnight in a 37°C incubator, 5% CO<sub>2</sub>.
- 3. The following day, replace the medium with 15 ml of pre-warmed, complete medium. **Note**: you do not need to add blasticidin in the completed medium. Optionally for long term culture to maintain cell line's genetic stability, you can add Blasticidin antibiotic in the medium at the final concentration as 10 µg/ml.
- 4. Incubate the cells and monitor cell density.
- 5. Passage cells (1:10 dilution) when the culture reaches 80-90% confluency.
- 6. Freeze cells at a density of 3  $\times$  10<sup>6</sup> cells/ml using 90% complete medium with 10% DMSO or cell recovery medium.

### **Complete medium**

DMEM (high glucose)

2mM L-glutamine

10% Fetal Bovine Serum (FBS)

0.1 mM Non-Essential Amino Acids (NEAA)

1% Pen-strep or 1x of Antibiotic-Antimycotic

## **Quality Control**

Each vial contains approximately  $3 \times 10^6$  cells with >95% viability before freeze. Cells are tested free of bacteria, viruses, mycoplasma.

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