

ACE2-3xFLAG-HEK293T stable cell line

Cat# CL0021 – 1 ml

Store at liquid nitrogen, ship with dry ice.

INTRODUCTION

ACE2 is also called ACEH and is called angiotensin converting enzyme 2. The protein encoded by this gene belongs to the angiotensin-converting enzyme family of dipeptidyl carboxydipeptidase, and is the functional receptor for SARS and HCoV-NL63 human coronavirus S glycoprotein. This ACE2-3xFLAG-HEK293T stable cell line expresses the ACE2-3xFLAG fusion protein, which can be used for ACE2 gene related research.

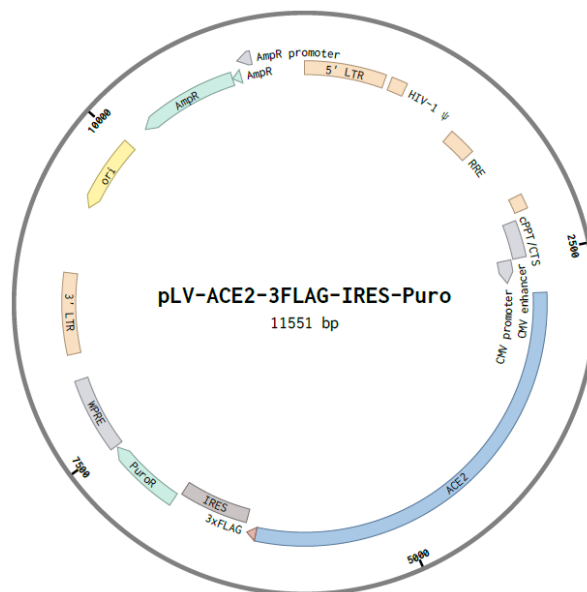
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No	Component	CL0021
AA	ACE2-3xFLAG-HEK293T frozen vial	1 ml (1 x 10 ⁷)

CONSTRUCTION

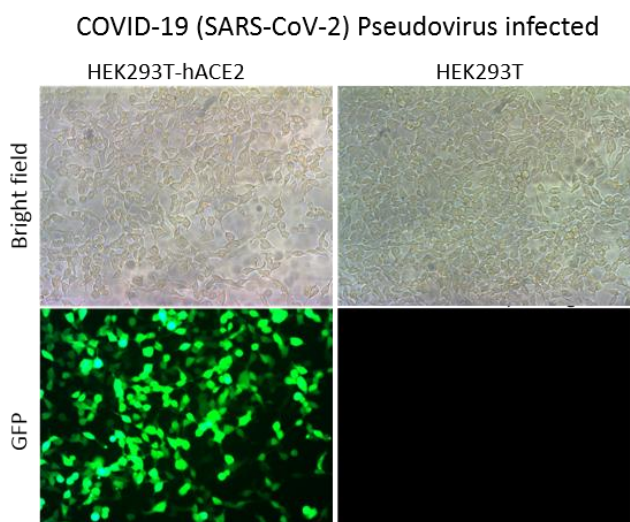
Stable Cell Line: The ACE2-3xFLAG-HEK293T stable cell line was obtained from lentivirus infected and multiple rounds of Puromycin screening. The lentivirus was packaged with pLV-ACE2-3FLAG plasmid, concentrated and purified, and then infected with HEK293T cells.

Vector Information



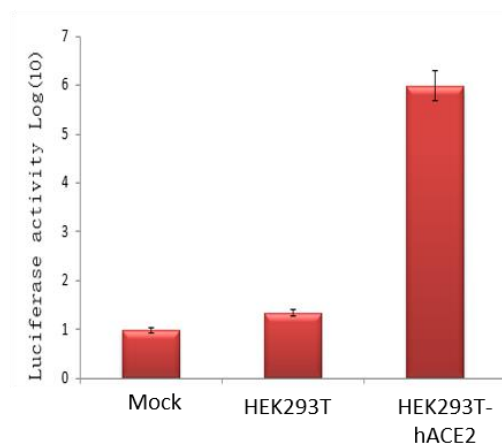
APPLICATIONS

GFP activity



Luciferase activity

COVID-19 (SARS-CoV-2) Pseudovirus infected



ACE2-3xFLAG-HEK293T stable cell infected by Pseudovirus-SARS-CoV-2 (ACE #PV031), can be observed the expression of green fluorescent protein and detected the activity of luciferase.

HANDLING PROCEDURE for FROZEN CELLS

1. Prepare and pre-warm Complete Growth Medium: DMEM+10 %FBS+ 1 %P/S + 3µg/ml puromycin
2. Thaw frozen cells rapidly (< 1 minute) in a 37°C water bath.
3. Dilute the thawed cells slowly with pre-warmed growth medium.
4. Plate thawed cells at high density to optimize recovery.

SUB-CULTURING

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca²⁺/Mg²⁺ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.05% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 to 2.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 10 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C.

Sub-cultivation ratio: A sub-cultivation ratio of 1:3 to 1:5 is recommended.

Medium renewal: Every 2 to 3 days

CYROPRESERVATION

Freeze medium: complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

CULTURE CONDITIONS

Temperature: 37°C

Atmosphere: air, 95%; carbon dioxide (CO₂)

Note :

1. Always use proper aseptic technique and work in a laminar flow hood.
2. Always wear personal protective equipment, including a face mask or goggles. Cryovials stored in liquid-phase present a risk of explosion when thawed.
3. Some freezing media contain DMSO, which is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment.

PRODUCT USE LIMITATION

These products are intended for research use only.