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Data Sheet

ACE2 – HeLa Recombinant Cell Line

Catalog #79958

Description

Recombinant clonal stable HeLa cell line constitutively expressing full length human ACE2, Genbank #NM_021804.3). Surface expression of ACE2 was confirmed by flow cytometry.

Background

Human Angiotensin converting enzyme 2 (ACE2), also known as ACEH, is an integral membrane protein found in the outer space of cells in the lungs, arteries, heart, kidney, and intestines. ACE2 serves as the entry point into cells for some coronaviruses, including the SARS-CoV-2 virus that is responsible for the COVID-19 pandemic.

Application

This cell line is useful for ACE2 binding assays, flow cytometry, or for screening ACE2 antibodies.

Format

Each vial contains ~ 2 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage

Store in liquid nitrogen immediately upon receipt.

Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination.

Cell Culture

Thaw Medium 1 (BPS Bioscience #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 1N (BPS Bioscience #79801): Thaw Medium 1 (BPS Bioscience #60187) and 0.5 µg/ml of Puromycin (InvivoGen, #ant-pr-1).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1N.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, and transfer to a tube containing 10 ml of Thaw Medium 1 (no puromycin). Spin down cells, resuspend cells in pre-warmed Thaw Medium 1 (no puromycin), transfer resuspended cells to a T25 flask and culture in 37°C CO_2 incubator. At first passage switch to Growth Medium 1N (contains puromycin). Cells should be split before they reach complete confluence.

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To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add Growth Medium 1N and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:5 to 1:10 weekly or twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1N and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Sequence

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMN
NAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIY
STGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNE
MARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNA
YPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKF
FVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHE
MGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL
LKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPA
SLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEP
WTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISLKSAL
GDKAYEWNDNEMYLFRSSVAYAMRQYFLKVKNQMILFGEEDVRVANLKPRISFNFFVTAPKNV
SDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPPNQPPVSIWLIVFGVVMGVIVVGIV
ILIFTGIRDRKKKNKARSGENPYASIDISKGENNPGFQNTDDVQTSF

Materials Required but Not Supplied

- HeLa growth medium or use
  Thaw Medium 1 (BPS Bioscience #60187): MEM supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- SARS-CoV-2 Spike Pseudotyped Lentivirus (Luc Reporter) (BPS Bioscience, #79942)
- 96-well tissue culture treated, white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HeLa cells using SARS-CoV-2 Spike pseudotyped lentivirus (Luciferase reporter). The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements.

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1. Day 1: Harvest ACE2-HeLa cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 1. Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: Add 5 µl of SARS-CoV-2 Spike Pseudotyped Lentivirus (Luc Reporter) into each well. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.

   Alternatively, seeding cells and the transduction can be performed at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 50 µl of fresh Thaw Medium 1 to each well.

   If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

3. Day 4-5, approximately 48-72 hours after transduction, prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 50 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. The transduction efficacy was determined by measuring the luciferase activity.

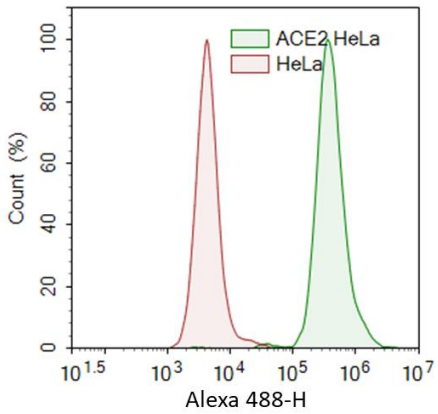

Figure 1. Expression of ACE2 validated by flow cytometry. ACE2-HeLa cells (green) or parental HeLa cells (red) were stained with anti-human ACE2 polyclonal goat IgG primary antibody (R&D Systems #AF933) and Alexa Fluor 488-conjugated rabbit anti-goat IgG secondary antibody (Thermo Fisher #A-21222). The ACE2 expression was analyzed by FACS.

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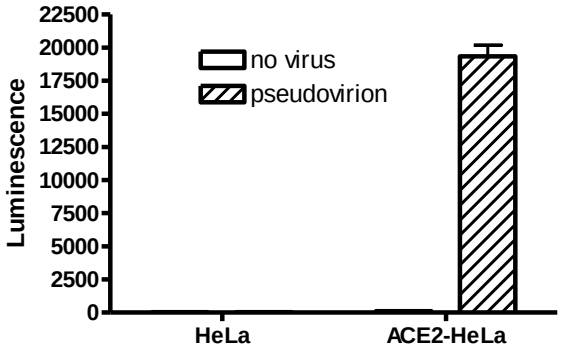

Figure 2. Transduction of ACE2-HeLa Cells using SARS-CoV-2 Spike Pseudotyped Lentivirus. Approximately 10,000 cells/well of ACE2-HeLa cells or HeLa parental cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter) (BPS Bioscience #79942). After 18 hours of transduction, the medium was changed to fresh HeLa growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The SARS-CoV-2 Spike pseudotyped lentivirus transduced ACE2-HeLa cells with much greater efficiency compared with HeLa parental cells, indicating the transduction is dependent upon ACE2 expression.

Related Products

Product	Cat. #	Size
ACE2 CHO Recombinant Cell Line	79959	2 vials
ACE2 HEK293 Recombinant Cell Line	79951	2 vials
ACE2 Lentivirus	79944	2 vials
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
ACE2, His-tag	11003-2	100 µg