Data Sheet
ACE2 – HEK293 Recombinant Cell Line
Catalog #AMS.79951

Description
Recombinant clonal stable HEK293 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 (#AMS.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 (#AMS.79801): Thaw Medium 1 (#AMS.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.

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 ration: 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

- HEK293 growth medium or use
  Thaw Medium 1 (#AMS.60187): MEM medium (Hyclone #SH30024.01)supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone#SV30010.01).
- Growth Medium 1N (#AMS.79801): Thaw medium 1 (#AMS.60187) and 0.5 µg/ml of Puromycin (InvivoGen, #ant-pr-1).
- Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter) (#AMS.79942)
- Bald lentiviral pseudovirion (Luciferase reporter) (#AMS.79943)
- Anti-SARS-CoV-2 Spike antibody (clone AM001414, #91361)
- Anti-ACE2 antibody (#AF933)
- Recombinant ACE2 protein (#AMS.11003)
- 96-well tissue culture treated, white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (#AMS.60690)

Assay Protocol

The following protocol is a general guideline for transducing ACE2-HEK293 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.

1. Day 1: Harvest ACE2-HEK293 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 (#AMS.60187). Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.

   To test anti-Spike antibody, preincubate 5 µl of the SARS-CoV-2 Spike pseudotyped lentivirus with 5 µl of diluted anti-Spike antibody for 30 minutes. After incubation, add 10 µl of virus/antibody mix into each well of the ACE2-HEK293 cells.

   To test anti-ACE2 antibody, add 5 µl of diluted anti-ACE2 antibody into each well of ACE2-HEK293 cells and incubate for 30 minutes. At the end of the incubation, add 5 µl of SARS-CoV-2 Spike pseudotyped lentivirus into each well.

   For control wells, the same number of ACE2-HEK293 cells were seeded, but no virus or antibody was added.

   Incubate the plates at 37°C with 5% CO_2 overnight.

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

If the tested antibody does not adversely affect the target cells, it is not necessary to change the medium on Day 3.

4. Day 4, approximately 48-60 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.

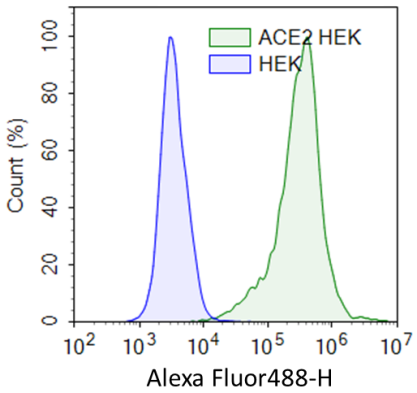
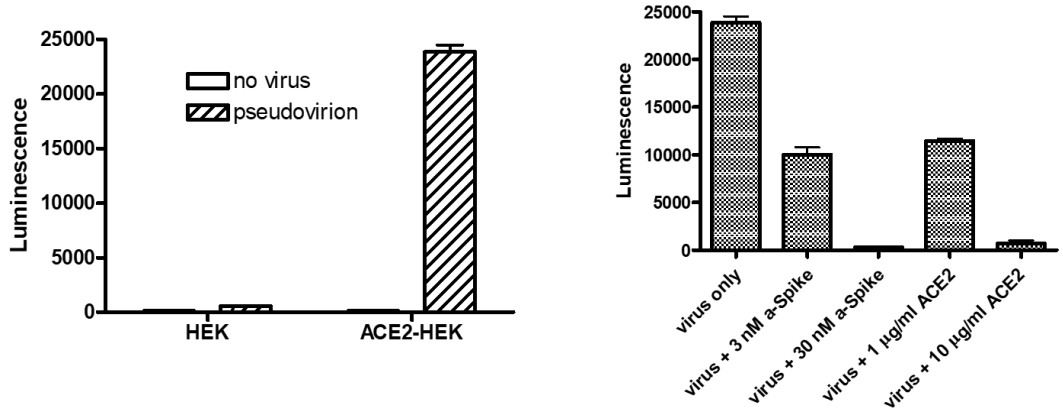


Figure 1. Expression of ACE2 validated by flow cytometry. ACE2-HEK293 cells (green) or parental HEK293 cells (blue) were stained with anti-human ACE2 polyclonal goat IgG primary antibody (#AF933) and Alexa Fluor 488 conjugated rabbit anti-goat IgGsecondary antibody (Thermo Fisher #A-21222). The ACE2 expression was analyzed by FACS.



A B

Figure 2. Transduction of ACE2-HEK293 Cells using SARS-CoV-2 Spike Pseudotyped Lentivirus. A. Approximately 10,000 cells/well of ACE2-HEK293 cells or HEK293 parental cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter) #AMS.79942). After 18 hours of transduction, the medium was changed to freshHEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (#AMS.60690) was added to cells to measure the luciferase activity. The SARS-CoV-2 Spike pseudotyped lentivirus transduced ACE2-HEK293 cells with much greater efficiency compared with HEK293 parental cells, indicating the transduction is dependent upon ACE2 expression. **B.** Approximately 10,000 ACE2-HEK293 cells/well were transduced with 10 µl/well of SARS-CoV-2 Spike pseudotyped lentivirus (Luc reporter) mixed with anti-Spike antibody (clone #AM001414, #91361) or recombinant ACE2 (#AMS.11003). After 18 hours of transduction, the medium was changed to fresh HEK growth medium (Thaw Medium 1). After 48 hours of transduction, ONE-Step Luciferase reagent (#AMS.60690) was added to cells to measure the luciferase activity.

Related Products

Product	Cat. #	Size
ACE2 CHO Recombinant Cell Line	AMS.79959	2 vials
ACE2 HeLa Recombinant Cell Line	AMS.79958	2 vials
ACE2 Lentivirus	AMS.79944	2 vials
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	AMS.79942	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	AMS.79943	500 µl x2
ACE2, His-tag	AMS.11003-2	100 µg
Thaw Medium 1	AMS.60187	100 ml
Growth Medium 1N	AMS.79801	500 ml

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