**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.

**Materials Provided**

| Components | Format |
|---|---|
| 2 vials of frozen cells | Each vial contains 2 x $10^6$ cells in 1 ml of 90% FBS, 10% DMSO |

**Host Cell**
HEK293

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

**Materials Required but Not Supplied**

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

*Materials Required for Cell Culture*

| Name | Ordering Information |
|---|---|
| Thaw Medium 1 | BPS Bioscience #60187 |
| Growth Medium 1N | BPS Bioscience #79801 |

*Materials Required for Cellular Assay*

| Name | Ordering Information |
|---|---|
| Thaw Medium 1 or HEK293 Growth Medium | BPS Bioscience #60187 |
| Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luciferase Reporter) | BPS Bioscience #79942 |
| Bald Lentiviral Pseudovirion (Luciferase Reporter) | BPS Bioscience #79943 |
| Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-1) | BPS Bioscience #100793 |
| Anti-ACE2 Antibody | R&D systems #AF933 |
| Recombinant ACE2 protein | BPS Bioscience #11003 |
| 96-well tissue culture treated, white clear-bottom assay plate | Corning #3610 |
| ONE-Step™ Luciferase Assay System | BPS Bioscience #60690 |

**Storage Conditions**

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

**Media Formulations**

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does not contain selective antibiotics. However, Growth Media does contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO2 using Growth Medium 1N.

*Media Required for 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).

*Assay Medium*: Thaw Medium 1 (BPS Bioscience #60184)

**Cell Culture Protocol**
**To thaw the cells:**

1. 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).
2. 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 CO2 incubator.
3. At first passage switch to Growth Medium 1N (contains puromycin).
4. Cells should be split before they reach complete confluence

**To passage the cells:**

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

**To freeze down the cells:**

8. Rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA.
9. 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.
10. Dispense 1 ml of cell aliquots into cryogenic vials.
11. Place vials in an insulated container for slow cooling and store at -80°C overnight.
12. Transfer to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

**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 (BPS Bioscience, #60187). Incubate cells at 37°C with 5% CO2 overnight.
2. Day 2: prepare serial dilutions of anti-Spike or anti-ACE2 antibody in Thaw Medium 1.

   **To test Spike S1 Neutralizing Antibody,** preincubate 5 µl of the SARS-CoV-2 Spike pseudotyped lentivirus with 5 µl of diluted Spike S1 Neutralizing 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% CO2 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.

**Validation Data**

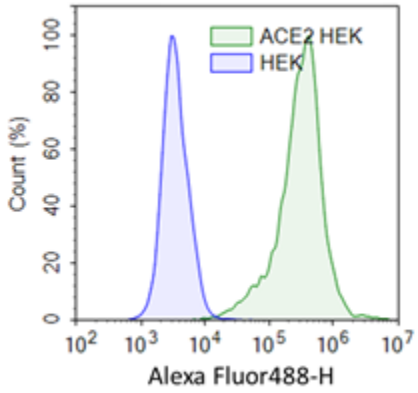
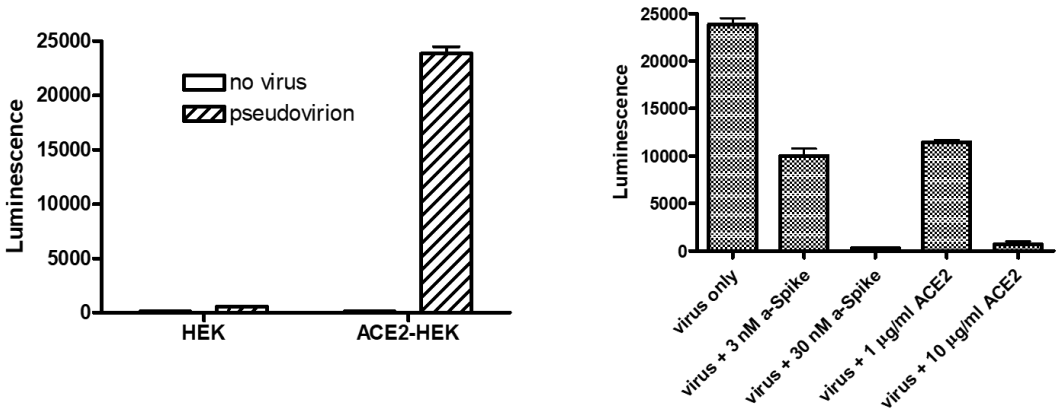



*Figure 1. Expression of ACE2 validated by flow cytometry.*

ACE2-HEK293 cells (green) or parental HEK293 cells (blue) were stained with anti-human ACE2 polyclonal goat IgG primary antibody (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.




**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) (BPS Bioscience #79942). 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 (BPS Bioscience #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 Spike S1 Neutralizing Antibody (SARS-CoV-2) (Clone: 414-1) (BPS Bioscience #100793) or recombinant ACE2 (BPS Bioscience, #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 (BPS Bioscience #60690) was added to cells to measure the luciferase activity.

**Sequence**

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMY
PLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESW
RSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAY
PSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLT
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KHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYC
DPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLALENVVGAKNMNVR
PLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYAMRQYFLKVKNQMILF
GEEDVRVANLKPRISFNFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPPNQPPVSIWLIVFGVVMGVI
VVGIVILIFTGIRDRKKKNKARSGENPYASIDISKGENNPGFQNTDDVQTSF

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**Troubleshooting Guide**

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| ACE2 CHO Recombinant Cell Line | 79959 | 2 vials |
| ACE2 HeLa Recombinant Cell Line | 79958 | 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 |
| Thaw Medium 1 | 60187 | 100 ml |
| Growth Medium 1N | 79801 | 500ml |