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Data Sheet

ACE2 - CHO Recombinant Cell Line

Catalog #79959

Description

Recombinant clonal stable CHO 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.

Host Cell

CHO K1 cell line, Chinese Hamster Ovary

Format

Each vial contains ~ 2 x 10⁶ 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 3 (BPS Bioscience, #60186): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3J (BPS Bioscience, #79974): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 5 µg/ml of Puromycin to ensure recombinant expression.

Cells should be grown at 37°C with 5% CO₂ using Growth Medium 3J.

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Recommended Culture Condition

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water bath, transfer to a tube containing 10 ml of **Thaw Medium 3 (no Puromycin)**, spin the cells down, remove the supernatant, and then re-suspend the cells in pre-warmed Thaw Medium 3 (**no Puromycin**). Then transfer the re-suspended cells to a T25 flask and culture in a 37°C CO₂ incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 (**no Puromycin**) and continue growing in a CO₂ incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. After the first passage, switch to **Growth Medium 3J** (contains 5 µg/ml Puromycin).

To passage the cells, rinse the cells with Phosphate Buffered Saline (PBS), detach the cells from the culture vessel with 0.25% Trypsin/EDTA, and add Growth Medium 3J and transfer to a tube. Next, spin the cells down, remove the supernatant, and then re-suspend the cells and seed appropriate aliquots of the cell suspension into new culture vessels. Suggested subcultivation ratios: 1:10 to 1:20 twice a week.

To freeze the cells down, rinse the cells with Phosphate Buffered Saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA. After detachment, add **Thaw Medium 3 (no Puromycin)** and count the cells, then transfer to a tube, spin the cells down, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10⁶ cells/ml. Dispense 1 ml of cell aliquots into each cryogenic vial. 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 passages.

Materials Required but Not Supplied

- Thaw Medium 3 (BPS Bioscience, #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- Growth Medium 3J (BPS Bioscience, #79974): Thaw Medium 3 (BPS Bioscience, #60186) plus 5 µg/ml of Puromycin (Takara, #631306) to ensure recombinant expression.
- Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter) (BPS Bioscience, #79942)
- Bald lentiviral pseudovirion (Luciferase reporter) (BPS Bioscience, #79943)
- Anti-SARS-CoV-2 Spike antibody (clone AM001414, Active motif, #91361)
- 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)

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Sequence

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMN
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MARANHYEDYGDYWRGDYEVNGVDGYDYSRGLIEDVEHTFEEIKPLYEHLHAYVRAKLMNA
YPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKF
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SLFHVSNDYSFIRYYTRTLYQFQFQEQALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEP
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GDKAYEWNENEMYLFRSSVAYAMRQYFLKVKNQMILFGEEDVRVANLKPRIFFVTAPKNV
SDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPNNQPPVSIWLIVFGVVMGVIVVGIV
ILIFTGIRDREKKNKARSGENPYASIDISKGENNPGFQNTDDVQTSF

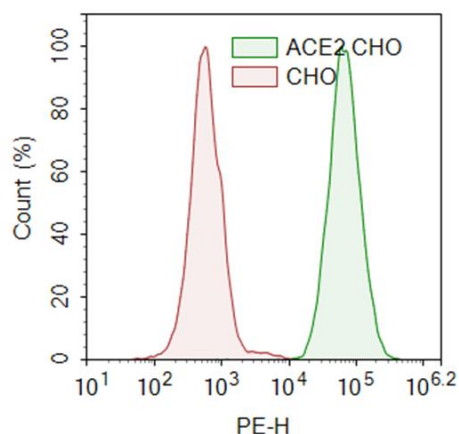


Figure 1. Expression of ACE2 validated by flow cytometry. ACE2-CHO cells (green) or parental CHO-K1 cells (red) were stained by Biotinylated Spike S1 (BPS Bioscience #100679) and PE conjugated Streptavidin (Biolegend #405204). The ACE2 expression was analyzed by FACS.

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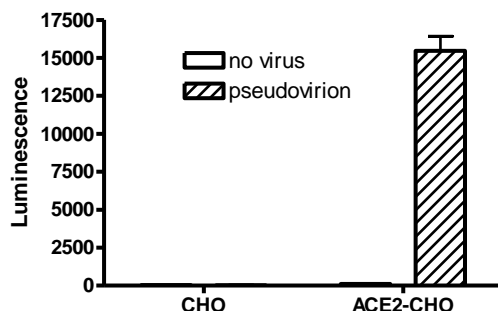


Figure 2. Transduction of ACE2-CHO Cells using SARS-CoV-2 Spike Pseudotyped Lentivirus. Approximately 10,000 cells/well of ACE2-CHO cells or CHO parental cells were transduced with 5 μ l/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter) (BPS Bioscience#79942). After 18 hours of transduction, the medium was changed to fresh CHO growth medium (Thaw Medium 3, BPS Bioscience #60186). 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-CHO with much greater efficiency compared with CHO parental cells, indicating the transduction is dependent upon ACE2 expression.

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Related Products

Product	Cat. #	Size
ACE2 HEK293 Recombinant Cell Line	79951	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 μ l x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 μ l x2

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