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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
TMPRSS2 – Vero E6 Recombinant Cell Line
Catalog #78081

Description
Recombinant clonal stable Vero E6 cell line constitutively expressing full length human TMPRSS2, Genbank #NM_005656.4.

Background
Human transmembrane serine protease 2 (TMPRSS2) is an enzyme primarily expressed by endothelial cells across the respiratory and digestive tracts. It is involved in viral entry and spread of coronaviruses including SARS-CoV-2, the virus that causes COVID19. Blocking TMPRSS2 could potentially be an effective clinical therapy for COVID-19.

Application
This cell line is useful for screening human TMPRSS2 inhibitors.

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 1P (BPS Bioscience, #78095): Thaw Medium 1 (BPS Bioscience, #60187) and 3 µg/ml of Puromycin (InvivoGen, #ant-pr-1).

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

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 1P (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 1P 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 1P 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 at least 10 vials of cells at early passage number for future use.

Materials Required but Not Supplied

- Assay Medium: Thaw Medium 1 (BPS Bioscience, #60187): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 1% Penicillin/Streptomycin.
- Growth Medium 1P (BPS Bioscience, #78095): Thaw medium 1 (BPS Bioscience, #60187) and 3 µg/ml of Puromycin (InvivoGen, #ant-pr-1).
- Vero E6 cells (ATCC #CRL-1586)
- Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter) (BPS Bioscience, #79942)
- Camostat mesylate (Sigma, #SML0057): 50 mM stock solution in water
- E-64d (Sigma, #E8640): 15 mM stock solution in methanol:water (1:1)
- 96-well tissue culture treated, white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)

Assay Protocol

1. Prepare serial dilutions (10x) of TMPRSS2 inhibitors in assay medium. Add 10 µl of diluted inhibitor into each well. For control wells, add just 10 µl of assay medium.

   Harvest TMPRSS2 Vero E6 cells from culture and seed cells at a density of 5,000-8,000 cells per well in 85 µl of assay medium into a white opaque 96-well microplate. Mix the cells and the inhibitor well by gentle pipetting. Incubate cells with the inhibitor at 37°C with 5% CO_2 for 1 hour.

2. Add 5 µl of the SARS-CoV-2 Spike pseudotyped lentivirus (BPS Bioscience, #79942) into each well. Gently mix the pseudovirus with the TMPRSS2 Vero E6/inhibitor mixture. Do not disturb the cells if they are starting attaching. Incubate the plate at 37°C with 5% CO_2 for ~48-60 hours.

   For control cells, seed the same number of TMPRSS2-Vero E6 cells but do not add virus or inhibitor.

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3. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 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 is determined by measuring the luciferase activity.

   Note: if the test compound interferes with the luciferase reading, it is recommended to remove the cell medium before adding the luciferase reagent.

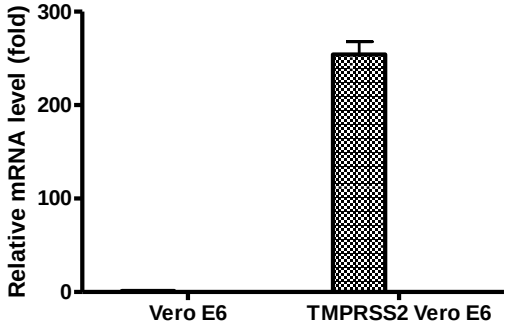

Figure 1. Expression of TMPRSS2 in TMPRSS2 Vero E6 cell line. Expression of TMPRSS2 in TMPRSS2 Vero E6 cell line was compared with that in parental Vero E6 cells by probe-based quantitative real-time PCR (Thermo Fisher# 4331182).

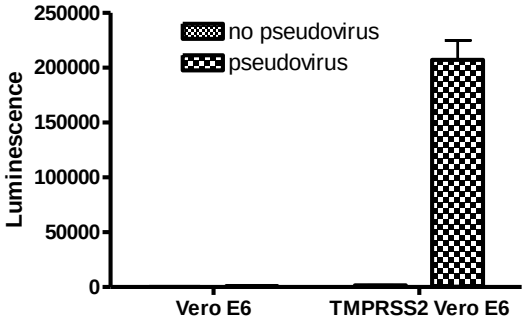

Figure 2. Enhanced infectivity of SARS-CoV-2 Spike pseudotyped lentivirus by TMPRSS2 expression in Vero E6 cells. Approximately 8,000 cells/well of TMPRSS2 Vero E6 cells or the parental Vero E6 cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #79942). 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 TMPRSS2 Vero E6 with much greater efficiency compared with parental Vero E6 cells, indicating the transduction is dependent upon TMPRSS2 expression.

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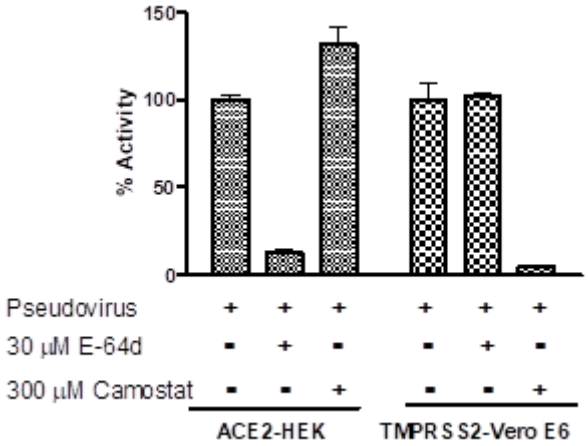

Figure 3. Inhibition of pseudovirus infection by protease inhibitors. Approximately 8,000 cells/well of ACE2-HEK293 cell line (BPS Bioscience, #79951) or TMPRSS2 Vero E6 cell line were treated with cysteine protease inhibitor E-64d or serine protease inhibitor camostat mesylate for one hour, and then transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #79942). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. Camostat mesylate efficiently blocked pseudovirus infection of TMPRSS2-Vero E6 cell, indicating that in this cell line, the pseudovirus employs TMPRSS2 for spike protein priming.

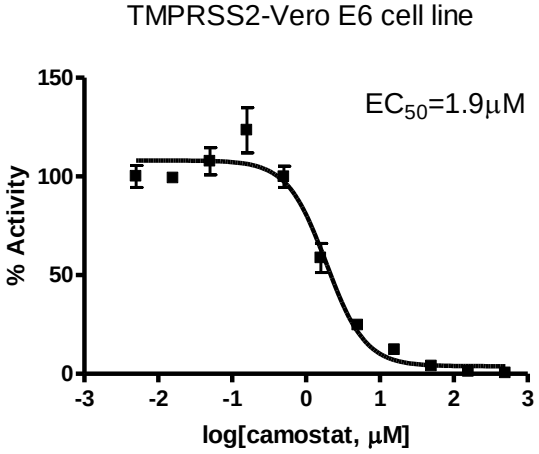

Figure 4. Inhibition of pseudovirus infection by camostat in TMPRSS2-Vero E6 cell line. Approximately 6,000 cells/well of TMPRSS2 Vero E6 cell line were treated with serially diluted camostat mesylate for one hour, and then transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luc reporter) (BPS Bioscience, #79942). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to cells to measure the luciferase activity. The wells without camostat treatment were set at 100% activity.

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Sequence
Genbank #NM_005656.4

MALNSGSPPAIGPYYENHGYQPENPYPAQPTVVPTVYEVHPAQYYPSPVPQYAPRVLTQASN
PVVCTQPKSPSGTVCTSKTKKALCITLTLGTFLVGAALAAGLLWKFMGSKCSNSGIECDSSGTC
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QGDSGGPLVTSKNNIWWLIGDTSWGSGCAKAYRPGVYGNVMVFTDWIYRQMRADG

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Related Products

Product	Cat. #	Size
ACE2 HEK293 Recombinant Cell Line	79951	2 vials
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942 -1	100 µl
SARS-CoV-2 Spike Pseudotyped Lentivirus (Luciferase Reporter)	79942 -2	500 µl x2
Bald Lentiviral Pseudovirion (Luciferase Reporter)	79943	500 µl x2
Thaw Medium 1	60187	100 ml
Growth Medium 1P	78095	500 ml
ACE2 HeLa Recombinant Cell Line	79958	2 vials
ACE2 Lentivirus	79944	2 vials
ACE2, His-tag	11003-2	100 µg