TMPRSS2 – Vero E6 Recombinant Cell Line

#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 COVID-19. Blocking TMPRSS2 could potentially be an effective clinical therapy for COVID-19.

Application

- Screen human TMPRSS2 inhibitors

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

Vero C1008 cells, Vero 76 clone, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1P	BPS Bioscience #78095

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Materials Used in Cellular Assays

Name	Ordering Information
H-CELL-100 Medium	Wisent Bio Products #001-035-CL
Spike (SARS-CoV-2) Pseudotyped Lentivirus (Luc Reporter)	BPS Bioscience #79942
E-64d	Sigma #E8640
Camostat mesylate	Sigma #SML0057
Gabexate mesylate	Sigma #SML2964
Boc-QAR-AMC Fluorogenic Peptide Substrate	R&D Systems #ES014
Vero E6 cell line	ATCC #CRL-1586
ACE2 – HEK293 cell line	BPS Bioscience #79951
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	
Fluorescence plate reader	

Storage Conditions

Cells are shipped in 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. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Penicillin/Streptomycin.

Growth Medium 1P (BPS Bioscience #78095):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, and 3 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Penicillin/Streptomycin.

Assay Medium: H-CELL-100 Medium (Wisent Bio Products, #001-035-CL)

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1 **(no Puromycin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1 **(no Puromycin)**.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 **(no Puromycin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium **1P (contains Puromycin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1P and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium **1P (contains Puromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:5 to 1:10 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1P and count the cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage.

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

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A. Validation Data

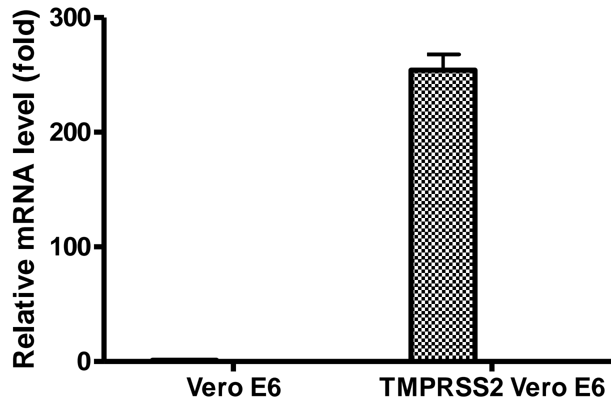

Figure 1. Expression of TMPRSS2 in TMPRSS2 Vero E6 cell line. Expression of TMPRSS2 in TMPRSS2 Vero E6 cell line was compared to expression in parental Vero E6 cells using probe-based quantitative real-time PCR (Thermo Fisher# 4331182).

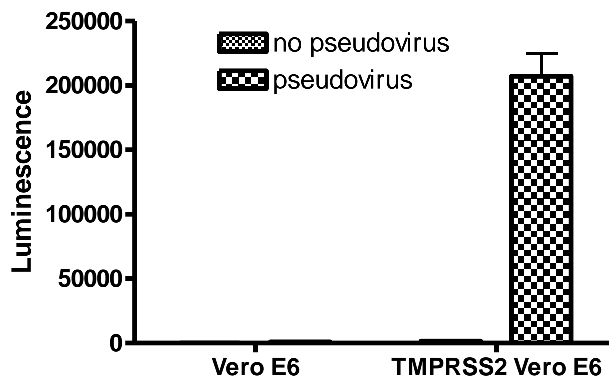

Figure 2. Enhanced infectivity of SARS-CoV-2 Spike pseudotyped lentivirus associated with TMPRSS2 expression in Vero E6 cells. Approximately 8,000 cells/well of TMPRSS2 Vero E6 cells or parental Vero E6 cells were transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luciferase reporter) (BPS Bioscience #79942). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience #60690) was added to cells The SARS-CoV-2 Spike pseudotyped lentivirus transduced TMPRSS2 Vero E6 with much greater efficiency compared to parental Vero E6 cells, indicating that transduction is dependent upon TMPRSS2 expression.

B. Functional characterization of TMPRSS2 Vero E6 Cell Line

The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)

a. Inhibition of SARS-CoV-2 Spike pseudovirus infection in TMPRSS2 Vero E6 cells by TMPRSS2 inhibitors

1. Prepare serial dilutions of TMPRSS2 inhibitors at concentrations 10-fold higher than the final desired concentration in Thaw Medium 1. Add the inhibitor to a white clear-bottom 96-well plate:

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- Add 10 µl of diluted inhibitor to the treated wells.

- Add 10 µl of Thaw Medium 1 to the untreated control wells.

2. Seed TMPRSS2 Vero E6 cells at a density of 5,000-8,000 cells per well in 85 µl of Thaw Medium 1 into the plate containing the inhibitor. 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.

3. Add 5 µl of the SARS-CoV-2 Spike pseudotyped lentivirus (BPS Bioscience #79942) to the treated wells and the untreated positive control wells. Add 5 µl Thaw Medium 1 to the uninfected control wells. Gently mix the pseudovirus with the TMPRSS2 Vero E6/inhibitor mixture. Do not disturb the cells if they are starting to attach. Incubate the plate at 37°C with 5% CO_2 for ~48-60 hours.

4. Perform luciferase assay using ONE-Step™ Luciferase Assay System (BPS Bioscience #60690), prepared according to the recommended instructions. Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. The transduction efficacy is determined by measuring the luciferase activity.

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

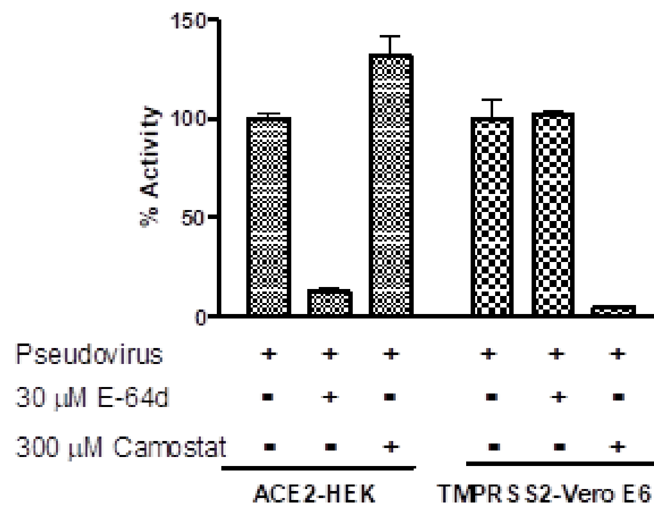

Figure 3: Inhibition of SARS-CoV-2 Spike pseudovirus infection by protease inhibitors in ACE2 HEK293 cells and TMPRSS2 Vero E6 cells. Approximately 8,000 cells/well of the ACE2 HEK293 cell line (BPS Bioscience #79951) or TMPRSS2 Vero E6 cell line were treated for one hour with cysteine protease inhibitor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

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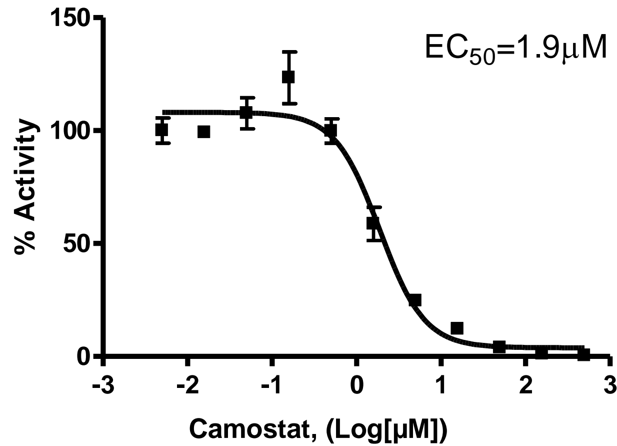

Figure 4: Inhibition of SARS-CoV-2 Spike pseudovirus infection by Camostat mesylate in TMPRSS2 Vero E6 cells. Approximately 6,000 cells/well of TMPRSS2 Vero E6 cells were treated with serially diluted Camostat mesylate for one hour, and then transduced with 5 µl/well of SARS-CoV-2-Spike pseudotyped lentivirus (Luciferase 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. Results are expressed as percent of control, defined as wells without Camostat treatment set at 100% activity.

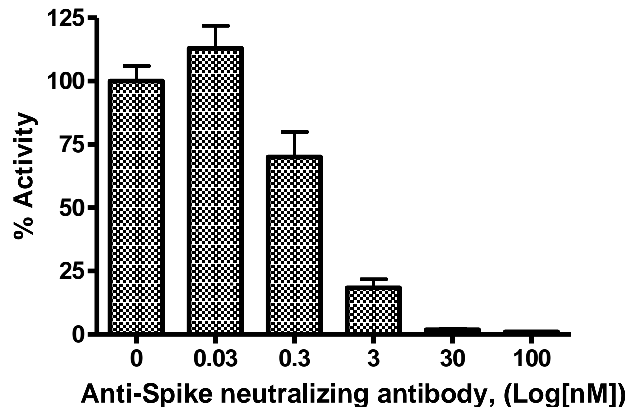

Figure 5: Neutralization of SARS-CoV-2 Spike speudovirus infection by anti-SARS-CoV-2 Spike antibody. Approximately 8,000 TMPRSS2 Vero E6 cells/well were transduced with 10 µl/well of SARS-CoV-2 Spike pseudotyped lentivirus (BPS Bioscience #79942) premixed with anti-Spike antibody (clone #AM002414, Active motif #91349). After 48 hours of transduction, ONE-Step Luciferase reagent (BPS Bioscience #60690) was added to measure the luciferase activity.

b. Measure of TMPRSS2 proteolytic activity using a fluorogenic substrate

Boc-QAR-AMC is an internally quenched substrate. Upon proteolysis, the substrate is cleaved to generate a highly fluorescent fragment. Therefore, fluorescence intensity increases proportionally to the activity of TMPRSS2.

This protocol uses H-CELL-100 Medium as assay medium.

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1. Seed TMPRSS2 Vero E6 cells at a density of ~12,000 cells per well into a clear 96-well plate in 100 µl Thaw Medium 1. Keep three wells without cells for determination of background fluorescence. Each condition should be performed in triplicate.

2. Incubate the plate at 37°C in a CO_2 incubator for 16 to 24 hours.

3. The next day, carefully remove Thaw Medium 1 from the cells and wash with 100 µl PBS.

4. Carefully remove PBS from the cells and replace with 50 µl of H-CELL-100 Medium.

5. Prepare a 100 µM Boc-QAR-AMC solution in H-CELL-100 Medium. The final concentration of substrate after addition to the cells will be 50 µM. Prepare enough of this solution as it will be used to dilute the test compounds in step 6.

6. Prepare a serial dilution of inhibitor (Camostat mesylate or Gabexate mesylate) at concentrations 2-fold higher than the final desired concentration, using the 100 µM Boc-QAR-AMC solution prepared in step 5. For an IC_{50} dose curve, we recommend a final dose range of 0.0004 to 100 µM Camostat mesylate or 0.006 to 400 µM Gabexate mesylate.

   - Add 50 µl of the inhibitor serially diluted in the Boc-QAR-AMC solution to the treated wells.

   - Add 50 µl of the Boc-QAR-AMC solution to the control "no inhibitor" wells.

   - Add 50 µl of H-CELL-100 Medium to internal control "cells only" wells.

   - Add 50 µl of H-CELL-100 Medium and 50 µl of the Boc-QAR-AMC solution to cell-free control wells (for determining background fluorescence)

   The final incubation volume is 100 µl.

7. Incubate the plate at 37°C in a CO_2 incubator for 16 to 24 hours.

8. The next day, without disturbing the cell monolayer, transfer 90 µl of medium to a clear-bottom black 96-well plate and equilibrate to room temperature for 30 minutes in the dark.

9. Measure fluorescence at an excitation of 360 nm and emission of 460 nm.

10. Data Analysis: Subtract the background fluorescence (cell-free control wells) from the fluorescence reading of all wells. The percent proteolytic activity of TMPRSS2 is the background-subtracted fluorescence of treated wells divided by the average background-subtracted fluorescence of the "no inhibitor" control wells, multiplied by 100.

$$Percent\ activity = \left(\frac{luminescence\ of\ stimulated\ cells - avg.background}{avg.luminescence\ of\ unstimulated\ cells - avg.background}\right)x100$$

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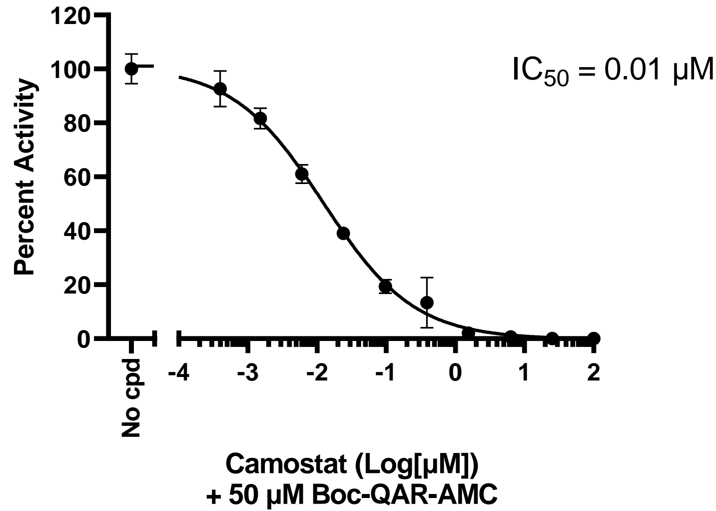

Figure 6: Inhibition of TMPRSS2 proteolytic activity by Camostat mesylate in TMPRSS2 Vero E6 cells. Cells were incubated with increasing concentrations of Camostat mesylate in the presence of 50 µM Boc-QAR-AMC for 24 hours before measuring the fluorescence of cleaved substrate present in the culture medium. Cleavage of Boc-QAR-AMC by TMPRSS2 was nhibited by Camostat mesylate in a dose-dependent fashion.

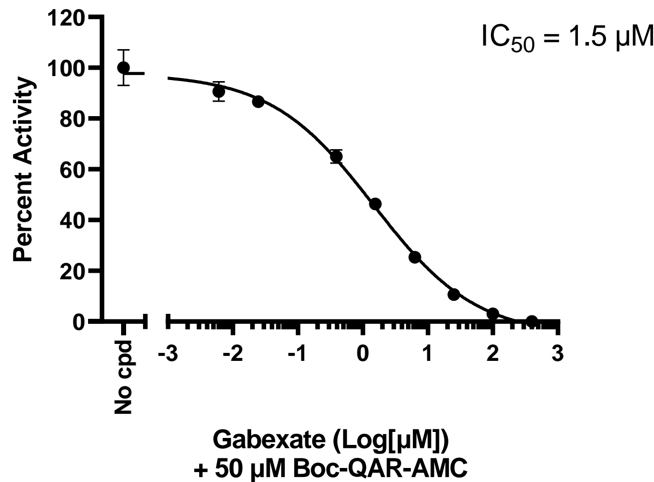

Figure 7: Inhibition of TMPRSS2 proteolytic activity by Gabexate mesylate in TMPRSS2 Vero E6 cells. Cells were incubated with increasing concentrations of Gabexate mesylate in the presence of 50 µM Boc-QAR-AMC for 24 hours before measuring the fluorescence of the cleaved substrate present in the culture medium. Cleavage of Boc-QAR-AMC by TMPRSS2 was inhibited by Gabexate mesylate in a dose-dependent fashion.

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Sequence

Genbank #NM_005656.4; UniProt #O15393

MALNSGSPPAIGPYYENHGYQPENPYPAQPTVVPTVYEVHPAQYYPSPVPQYAPRVLTQASNPVVCTQPKSPSGTVCTSKTKKA
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NLITPAMICAGFLQGNVDSCQGDSGGPLVTSKNNIWWLIGDTSWGSGCAKAYRPGVYGNVMVFTDWIYRQMRADG

References

Shapira T, *et al.* (2022) *Nature* **605**, 340-348

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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 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	100 µl/500 µl x2
TMPRSS2 Lentivirus	78011	Several sizes
TMPRSS2 Fluorogenic Assay Kit	78083	96 reactions

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