Description

The Transmembrane protease, serine 2 (TMPRSS2) Fluorogenic Assay Kit is provided in a 96-well format with purified TMPRSS2, TMPRSS2 Fluorogenic Substrate, and TMPRSS2 assay buffer for 96 reactions. The TMPRSS2 Fluorogenic Assay is a homogeneous assay that takes advantage of a specific fluorogenic substrate which emits fluorescence upon cleavage by the protease. Only one step is required to assess TMPRSS2 activity: TMPRSS2 protease is incubated with the fluorogenic substrate and fluorescence is measured using a plate reader (λex = 383 nm, λem = 455 nm). Camostat, a known TMPRSS2 inhibitor, is supplied as a protease inhibitor control.

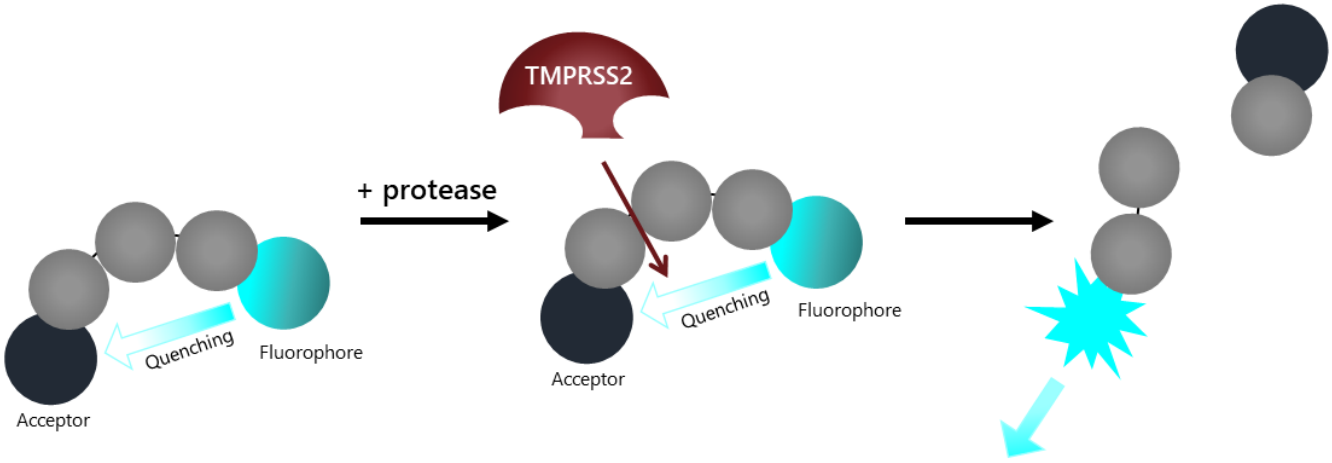

Figure: Illustration of the principle behind the fluorogenic TMPRSS2 protease assay. The peptide substrate is labeled with a fluorophore on one end and an acceptor on the other end. The fluorescence emitted by the donor fluorophore is quenched due to the proximity of the acceptor in the intact peptide. The protease cleaves the peptide and generates a highly fluorescent peptide fragment.

Background

TMPRSS2 is a serine protease that facilitates SARS-CoV-2 particle entry into host cells via priming of the viral protein Spike. Its inhibition blocks the fusion of the virus with the plasma membrane after Spike interacts with human receptor Angiotensin-converting enzyme 2 (ACE2), restricting SARS-CoV-2 viral entry, therefore TMPRSS2 is an important therapeutic target for the treatment of COVID-19.

The protein may also play a role in prostate cancer because its gene is frequently altered in tumor cells. Indeed, TMPRSS2-ERG and TMPRSS2-ETV1 fusions are frequent, with TMPRSS2-ERG present in approximately 40 to 80% of human prostate tumors. ETS-related gene (ERG) is a transcription factor that regulates the expression of genes involved in embryonic development, cell proliferation and differentiation, and apoptosis, acting as an oncogene. Alternatively, the tumor suppressor function of TMRSS2 is disrupted since the fusion genes arise from deletions that eliminate the TMPRSS2 coding region while juxtaposing its androgen-inducible promoter and the open reading frame of ERG. Overexpression of ERG contributes to development of androgen-independence in prostate cancer through disruption of androgen receptor signaling.

Application(s)

Study enzyme kinetics and screen small molecular inhibitors in high throughput (HTS) applications

Supplied Materials

Catalog #	Name	Amount	Storage
	TMPRSS2*	15 µg	-80°C
78047	TMPRSS2 Fluorogenic Substrate (5 mM)	10 µl	-20°C ***(Protect from light)***
78048	1x TMPRSS2 Assay Buffer	5 ml	-20°C
78049	Camostat	500 µg	-80°C
79685	96-well black plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube*

Materials Required but Not Supplied

- Fluorescent plate reader capable of reading fluorescence at λex = 383±15 nm, λem = 455±15 nm
- Adjustable micropipettor and tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%. Higher DMSO concentrations can significantly decrease the activity of the enzyme.

Assay Protocol

- All samples and controls should be tested in duplicate.
- A "Blank" should be included to determine background fluorescence
- TMPRSS2 inhibitor Camostat is included as an internal control

1. Thaw **TMPRSS2** on ice. Briefly spin the tube containing the enzyme to recover the full contents of the tube. If the assay plate is going to be used more than once, prepare enough enzyme for your portion of the assay and aliquot TMPRSS2 into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

   Note: TMPRSS2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots more than once and do not re-use the diluted enzyme.

2. Dilute **TMPRSS2** in 1X TMPRSS2 Assay Buffer at 5 ng/µl (150 ng/reaction). Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

3. Reconstitute the 500 µg of Camostat with 20 µl of distilled water (50 mM). Using a serial dilution, dilute Camostat 1000-fold with 1X TMPRSS2 Assay Buffer (50 µM). For example, dilute 10-fold by adding 180 µl of Assay Buffer to 20 µl of 50 mM Camostat, then dilute 100-fold by adding 990 µl of Assay Buffer to 10 µl of the previous 10-fold dilution.

4. Add 10 µl of Camostat (50 µM) to the wells labeled "Inhibitor Control." The final concentration of Camostat in the assay will be 10 µM.

5. Prepare the Test Inhibitor (10 µl/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.
   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the Assay Buffer, 5-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 20-fold in Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.
   Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in Assay Buffer to keep the concentration of DMSO constant.
   For positive and negative controls, prepare 5% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

6. Add 30 µl of the diluted **TMPRSS2 (5 ng/µl)** to all wells except for "Blank". Add 30 µl of Assay Buffer to the wells labeled as "Blank".

7. Incubate for 30 minutes at room temperature.

8. Thaw the **TMPRSS2 Fluorogenic Substrate** (5 mM) at room temperature.

Note: Protect this substrate from direct exposure to light!

9. Dilute the TMPRSS2 Fluorogenic Substrate (5 mM) 100-fold with 1x TMPRSS2 Assay buffer (50 µM). If the assay plate will be used more than once, dilute only enough to perform your assay.

10. Initiate the reaction by adding 10 µl of diluted TMPRSS2 Fluorogenic Substrate (50 µM) to all wells. The final concentration of TMPRSS2 Fluorogenic Substrate in the assay is 10 µM.

Component	Blank	Positive Control	Test Inhibitor	Camostat
1x TMPRSS2 Assay Buffer	30 µl	-	-	-
Test Inhibitor	-	-	10 µl	-
Camostat	-	-	-	10 µl
Diluent Solution	10 µl	10 µl	-	-
TMPRSS2 enzyme (5 ng/ µl)	-	30 µl	30 µl	30 µl
TMPRSS2 Substrate (50 µM)	10 µl	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

11. Protect the plate from light by covering it with aluminum foil. Incubate the plate for ten minutes at room temperature.

12. Read fluorescence at λex = 383 (±10) nm and λem = 455 (±10) nm. Alternatively, kinetic measurements can be performed for up to 30 minutes at 2 to 5-minute intervals. The "Blank" value is subtracted from all measurements. Note: Make sure the plate is not exposed to direct light.

Example Results

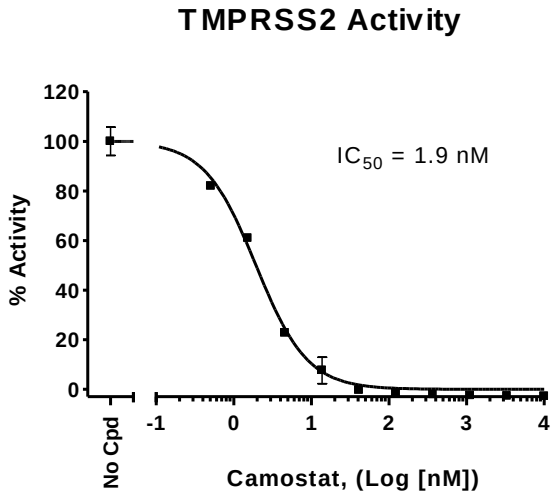

Figure 1: Inhibition of TMPRSS2 protease activity by Camostat.
Protease activity was measured in the presence of increasing inhibitor concentrations using the TMPRSS2 Fluorogenic Assay Kit. The "Blank" value was subtracted from all other values. Results are expressed as the percentage of control (protease activity in the absence of inhibitor, set at 100%).
For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

1. Shapira T, *et al*. A TMPRSS2 inhibitor acts as a pan-SARS-CoV-2 prophylactic and therapeutic. *Nature.* 2022; **605**: 340-348.
2. Tomlins SA, *et al*. Role of the TMPRSS2-ERG gene fusion in prostate cancer. *Neoplasia* 2008; **10(2)**: 177-188.

Related Products

Products	*Catalog #*	*Size*
TMPRSS2 Lentivirus	78011	Various sizes
TMPRSS2 Vero E6 Recombinant Cell Line	78081	2 vials
3CL Protease (SARS-CoV-2) Assay Kit	79955	96 reactions