SensoLyte® 520 TMPRSS2 Activity Assay Kit *Fluorimetric*

Catalog #	AS-72275
Kit Size	100 Assays (96-well plates)

- ***Convenient Format:*** All essential assay components are included.
- ***Optimized Performance:*** Optimal conditions for the detection of TMPRSS2 activity.
- ***Enhanced Value:*** It provides ample reagents to perform 100 assays in a 96-well plate format.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom, 96-well plate with non-binding surface.

Component	Description	Quantity
Component A	TMPRSS2 substrate, Ex/Em=490/520 nm upon cleavage	3 mM, 55 µL
Component B	Rh110, fluorescence reference standard, Ex/Em=490/520 nm	3 mM, 10 µL
Component C	Recombinant Human TMPRSS2 Protein	10 µL
Component D	2X Assay Buffer	20 mL
Component E	Inhibitor	100 µM, 15 µL

- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.

Storage and Handling

- Store Components A, B, and E at -20°C. Component C at -80°C.
- Keep Components A and B away from light.
- For convenience, Components D can be stored at 4°C.

Introduction

TMPRSS2 is part of the type 2 transmembrane serine protease (TTSP) family, which includes 19 trypsin-like serine proteases.[1] These enzymes are primarily found on cell surfaces and play critical roles in various biological processes. TMPRSS2 (transmembrane protease, serine 2) is indeed a crucial factor in the entry of certain viruses, including SARS-CoV, MERS-CoV, and various influenza viruses like H7N9 and H1N1.[2] Targeting TMPRSS2 could provide a promising antiviral strategy, as inhibiting its activity may block the viral entry process. Research is ongoing to develop specific inhibitors that can effectively disrupt TMPRSS2 function without adversely affecting normal cellular processes. This approach could pave the way for new treatments for both coronavirus and specific influenza infections, enhancing our ability to manage these viral diseases.[3] TMPRSS2 is also known to be highly expressed in metastatic prostate cancer and is regulated by androgen receptor signaling, making it a potentially valuable target to study tumor growth, metastasis and treatment of the disease.[4]

The TMPRSS2 Assay Kit is an assay for screening of enzyme inhibitors or for continuous assay of TMPRSS2 activity using a fluorogenic substrate. Upon cleavage by TMPRSS2, this substrate generates the Rh110 (Rhodamine 110) fluorophore which has a bright green fluorescence and can be detected at excitation/emission=496 nm/520 nm. The longer wavelength spectra and higher extinction coefficient of Rh110 provide greater sensitivity and less interference from other reaction components.

Protocol

Note 1: Please use protocol A or B based on your needs.
Note 2: For standard curve, please refer to Appendix II (optional).

Protocol A. Screening TMPRSS2 inhibitors using a Recombinant Human TMPRSS2 Protein.

1. Prepare working solutions.

1.1. 1X assay buffer: Add 10 ml of 2X Assay Buffer (Component D) to 10 mL deionized water. Prepare fresh 1X assay buffer for each experiment.

1.2. TMPRSS2 Rh110 substrate solution: Dilute substrate (Component A) 100-fold in assay buffer. Refer to Table 1.

Table 1. TMPRSS2 Rh110 substrate solution for one 96-well plate (100 assays).

Components	Volume
TMPRSS2 Rh110 substrate (Component A)	50 µL
1X Assay buffer	4.95 mL
Total volume	5 mL

1.3. TMPRSS2 diluent: Dilute Recombinant Human TMPRSS2 Protein solution (Component C) 400-fold in 1X assay buffer. This amount of enzyme is enough for a full 96-well plate. If not using an entire plate, adjust the amount of enzyme to be diluted accordingly.

Note: TMPRSS2 is highly sensitive to freeze/thaw cycles. Do not vortex enzyme solutions. Prepare enzyme diluents immediately before use. Prolonged storage, or agitation of the diluted enzyme will cause enzyme denaturation. Store on ice.

1.4. Inhibitor (Camostat): Dilute the 100 μM inhibitor solution (Component E) 1:100 in assay buffer. The diluted Camostat solution has a concentration of 1 μM. Add 10 μl of the diluted Camostat into each of the inhibitor control well.

2. Set up the enzymatic reaction.

2.1 Add test compounds and diluted enzyme solution to the microplate wells. The suggestedvolume of enzyme solution for a 96-well plate is 40 μL/well and test compound is 10 μL/well.

2.2 Simultaneously set up the following control wells, as deemed necessary:

- Positive control contains the enzyme without test compound.
- Inhibitor control contains Recombinant Human TMPRSS2 Protein and Camostat.
- Vehicle control contains Recombinant Human TMPRSS2 Protein and vehicle used in delivering test compound (e.g. DMSO, concentration not to exceed 1%).
- Test compound control contains assay buffer and test compound. Some test compounds have strong autofluorescence and may give false results.
- Substrate control contains assay buffer.

2.3 Use the assay buffer to bring the total volume of all controls to 50 μL.

3. Run the enzymatic reaction.

3.1 Add 50 μL of TMPRSS2 substrate solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 min for 60 min at 37ºC.
- For end-point reading: Incubate the reaction at 37ºC for 60 min. Keep plate from direct light. Then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

3.3 For methods of data analysis: Refer to Appendix I.

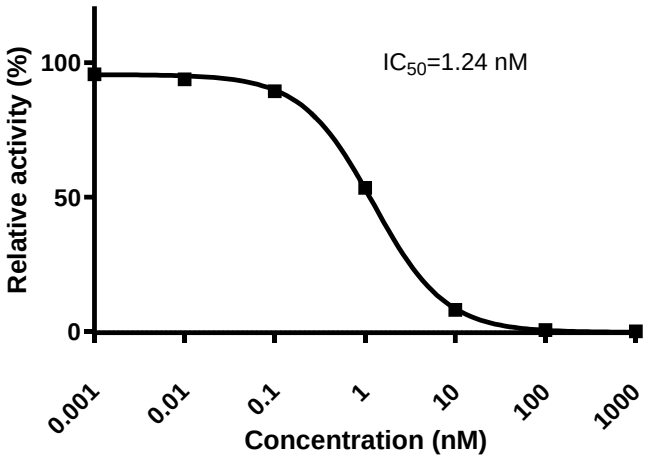

Figure 1. Inhibition of TMPRSS2 activity by Camostat as measured with SensoLyte® 520 TMPRSS2 Assay Kit.

Protocol B. Measuring TMPRSS2 activity in biological samples.

1. Prepare working solutions.

Note: Bring all kit components until thawed to room temperature before starting the experiments.

1.1 TMPRSS2 substrate solution: Dilute substrate (Component A) 100-fold in 2X assay buffer. Refer to Table 2. If not using the entire plate, adjust the amount of substrate to be diluted accordingly.

Table 2. TMPRSS2 substrate solution for one 96-well plate (100 assays)

Components	Volume
TMPRSS2 substrate (Component A)	50 µL
2X Assay buffer (Component D)	4.95 mL
Total volume	5 mL

1.2 TMPRSS2 diluent: If you use purified TMPRSS2 Protein as a positive control, dilute TMPRSS2 protein solution (Component C) 500-fold in 1X assay buffer (Refer to Protocol A, step 1.1 for preparation). Add 50 µl of the diluted enzyme into each of the positive control well.

Note 1: TMPRSS2 is highly sensitive to freeze/thaw cycles. Do not vortex enzyme solutions. Prepare enzyme diluents immediately before use. Prolonged storage, or agitation of the diluted enzyme will cause enzyme denaturation. Store on ice.
Note 2: For positive control use substrate solution diluted in 1X assay buffer as described in Protocol A, step 1.2.

2. Set up enzymatic reaction.

2.1 Add 50 µL/well (96-well plate) of TMPRSS2 containing biological sample.

2.2 Set up the following control wells at the same time, as deemed necessary:

- Positive control contains purified Recombinant Human TMPRSS2 Protein.
- Negative control contains biological sample without TMPRSS2 protein.
- Substrate control contains deionized water.

2.3 Using the assay buffer (Component D), bring the total volume of all controls to 50 µL (96-well plate).

3. Run the enzymatic reaction.

3.1 Add 50 µL/well (96-well plate) of TMPRSS2 substrate solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 min. for 30 to 60 min at 37 ºC.
- For end-point reading: Incubate the reaction at 37 ºC for 60 min. Keep plate from direct light. Then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

3.3 For methods of data analysis: Refer to Appendix I.

Appendix I. Data Analysis

- The fluorescence reading from the substrate control well is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells containing substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).
- For endpoint analysis:
  - Plot data as RFU versus concentration of test compounds.
  - A variety of data analyses can be done, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

Appendix II. Instrument Calibration

- Rh110 fluorescence reference standard: Dilute 3 mM Rh110 fluorescence reference standard (Component B) to 30 µM in assay buffer. Do 2-fold serial dilutions to get concentrations of 15, 7.5, 3.75, 1.875, 0.938, 0.469 µM and include 0 µM as an assay buffer blank. Add 50 µL/well of these serially diluted fluorescence reference solutions.
- Add 50 µL/well of the diluted TMPRSS2 substrate solution (refer to Protocol A, step 1.1 for preparation).

  Note: The TMPRSS2 substrate solution is added to the fluorescence reference standard to correct for the absorptive quenching by the FRET peptide. If multiple concentrations of substrate are used, this step must be performed for each concentration.
- Measure the fluorescence intensity of the reference standard and substrate control wells at Ex/Em=490 nm/520 nm. Use the same setting of sensitivity as used in the enzyme reaction.

- Plot the fluorescence reference standard curve as RFU (relative fluorescent units) versus concentration as shown in Figure 2.
- The final concentrations of fluorescence reference standard are 15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234 and 0 μM. This reference standard is used to calibrate the variation of different instruments and different experiments. Since the proteolytic cleavage of the Rh110 substrate consists of two steps, with both the intermediate and final products having fluorescence, the Rh110 reference standard cannot serve as an indicator of the amount of enzymatic reaction final product.

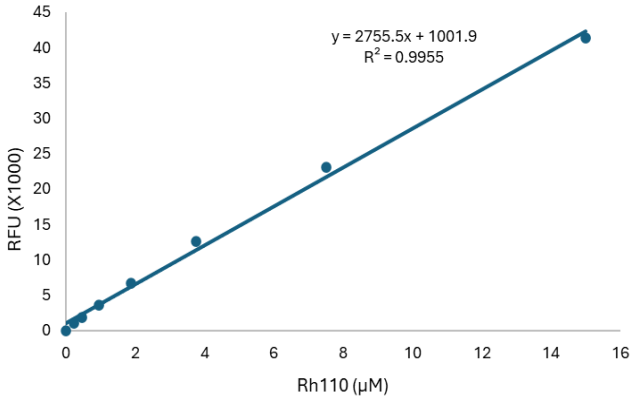

Figure 2. Rh110 fluorescence reference standard. Rh110 fluorescence was serially diluted in assay buffer, containing TMPRSS2 substrate, and the fluorescence recorded at Ex/Em=490 nm/520 nm (SpectraMax M^{5e}, Molecular Devices).

References

1. Fraser, Bryan J., et al. Nature chemical biology 18.9 (2022): 963-971.
2. Wettstein, Lukas, Frank Kirchhoff, and Jan Münch. International Journal of Molecular Sciences 23.3 (2022): 1351.
3. Shen, Li Wen, et al. Biochimie 142 (2017): 1-10.
4. Lucas, Jared M., et al. Cancer discovery 4.11 (2014): 1310-1325.