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# Data Sheet

## *TMPRSS2 Fluorogenic Assay Kit*

**Catalog # 78083**
**Size: 96 reactions**

**BACKGROUND:** Transmembrane protease, serine 2 is an enzyme that belongs to the serine protease family. It has been implicated as a target in prostate cancer. TMPRSS2 facilitates SARS-CoV-2 particle entry into host cells via S protein priming, and its inhibition blocks virus fusion with angiotensin-converting enzyme 2 (ACE2). This, in turn, restricts SARS-CoV-2 viral entry, making TMPRSS2 an important therapeutic target.

**DESCRIPTION:** The *TMPRSS2 Fluorogenic Assay Kit* is provided in a convenient 96-well format, with purified TMPRSS2, TMPRSS2 Fluorogenic Substrate, and TMPRSS2 assay buffer for 96 enzymatic reactions. The key to the *TMPRSS2 Fluorogenic Assay Kit* is the fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for TMPRSS2 reactions. A sample containing TMPRSS2 is incubated in a reaction mixture with the fluorogenic substrate and fluorescence ($\lambda_{ex}$ = 383 nm, $\lambda_{em}$ = 455 nm) is measured using a plate reader. Camostat is supplied as a protease inhibitor control.

**COMPONENTS:**

| Catalog # | Component | Amount | Storage | |
|---|---|---|---|---|
| | TMPRSS2 | 15 µg | -80°C | ***Avoid freeze/ thaw cycles!*** |
| 78047 | TMPRSS2 Fluorogenic Substrate (5 mM) | 10 µl | -20°C | |
| 78048 | 1X TMPRSS2 Assay Buffer | 5 ml | -20°C | |
| 78049 | Camostat | 500 µg | -80°C | |
| 79685 | 96-well black plate | 1 | Room temp. | |

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**
Fluorescent microplate reader capable of reading fluorescence at $\lambda_{ex}$ = 383±15 nm, $\lambda_{em}$ = 455±15 nm
Adjustable micropipettor and tips
Rotating or rocker platform

**APPLICATIONS:** Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

**STABILITY:** One year from date of receipt when stored as directed.

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**REFERENCES:**

1. Tomlins, S. A., *et al*. 2008. "Role of the TMPRSS2-ERG gene fusion in prostate cancer." *Neoplasia* **10 (2):** 177-188.
2. Hoffmann, M., *et al.,* 2020. "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor". *Cell* **181:** 271–280.

**ASSAY PROTOCOL:**

***All samples and controls should be tested in duplicate.***

**Step 1:**

1) Thaw **TMPRSS2** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **TMPRSS2** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: TMPRSS2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
2) Dilute **TMPRSS2** in **1X TMPRSS2 Assay Buffer** at 5 ng/µl (150 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
3) Re-constitute the 500 µg of **Camostat** with 20 µl of water (50 mM). Dilute **Camostat** 1000-fold with assay buffer (50 µM). Add 10 µl to the wells labeled "Inhibitor Control." The final concentration of **Camostat** in the assay will be 10 µM.
4) Dispense 10 µl of Inhibitor solution to each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 10 µl of 5% DMSO in buffer (Inhibitor buffer). Maintain the same level of DMSO in the controls as the test sample(s).

   *Note: Final DMSO concentration must be ≤2%. Higher DMSO level can significantly decrease the enzyme activity. For example, to test an inhibitor at 10 µM that is dissolved in 100% DMSO, dilute 1 mM inhibitor with buffer to make 50 µM inhibitor in 5% DMSO (aqueous). Then, use 10 µl of the 50 µM solution for the 50 µl assay.*

| | Positive Control | Inhibitor Control | Test Inhibitor | Blank |
|---|---|---|---|---|
| Camostat | – | 10 µl | – | – |
| Test Inhibitor | – | – | 10 µl | – |
| Inhibitor buffer (5% DMSO in buffer) | 10 µl | – | – | 10 µl |
| 1x TMPRSS2 Assay Buffer | - | | - | 30 µl |
| TMPRSS2 (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** |

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5) Add 30 µl of diluted **TMPRSS2** (5 ng/µl) to all wells except for "Blank". For "Blank," add 30 µl of **1x TMPRSS2 Assay buffer**. Incubate plate for 30 minutes at room temperature.

6) Begin thawing **TMPRSS2 Fluorogenic Substrate (5 mM)** at room temperature. *Note: Protect this substrate from direct exposure to light!*

**Step 2:**

***Note: Protect TMPRSS2 Fluorogenic Substrate from direct exposure to light!***

7) Dilute **TMPRSS2 Fluorogenic Substrate (5 mM)** 100-fold with **1x TMPRSS2 Assay buffer** to 50 µM.

8) Initiate reactions by adding 10 µl of **TMPRSS2 Fluorogenic Substrate** (50 µM) to all wells. Final concentration of **TMPRSS2 Fluorogenic Substrate** is 10 µM.

9) Protect plate from light by covering with aluminum foil. Incubate plate for ten minutes at room temperature.

**Step 3:**

10) Read fluorescence at $\lambda_{ex}$ = 383 (±15) nm and $\lambda_{em}$ = 455 (±15) nm. Alternatively, kinetic measurements can also be monitored for up to 30 minutes at 2-5 minute intervals. "Blank" value is subtracted from all measurements. *Note: Make sure the wells are not exposed to direct light.*

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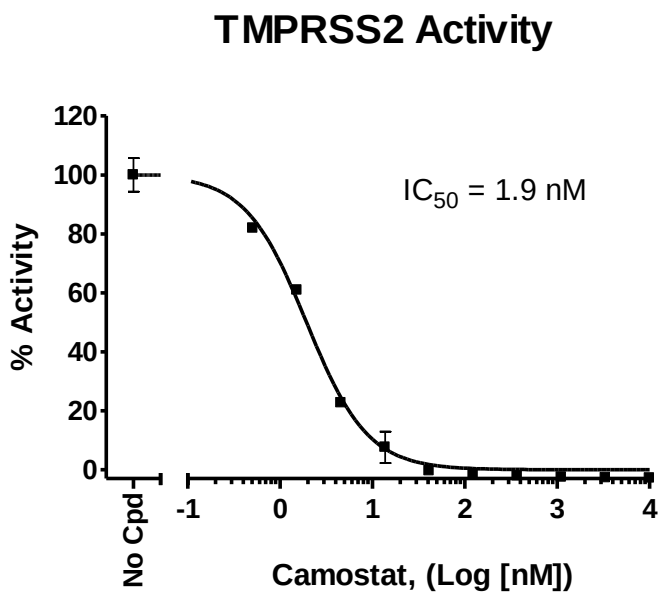
**Example of Assay Results:**




Inhibition of TMPRSS2 by Camostat (BPS Bioscience, #78049), measured using the *TMPRSS2 Fluorogenic Assay Kit* (BPS Bioscience #78083). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

**Related Products**

| Product | Cat. # | Size |
|---|---|---|
| Furin Protease Assay Kit | 78040 | 96 rxns. |
| 3CL Protease, Untagged (SARS-CoV-2) Assay Kit | 78042-1 | 96 rxns. |
| 3CL Protease, Untagged (SARS-CoV-2) Assay Kit | 78042-2 | 384 rxns. |
| 3CL Protease (SARS-CoV-2), no tag | 100823-1 | 50 µg |
| 3CL Protease (Mpro) (SARS-CoV-2) | 100755 | 50 µg |
| 3CL Protease (SARS-CoV-2) Assay Kit | 79955 | 96 rxns. |
| 3CL Protease, His-tag (SARS-CoV-1) | 100807 | 100 µg |
| 3CL Protease (SARS-CoV-1) Assay Kit | 78015 | 96 rxns |

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