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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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 West, Ste. B
San Diego Ca 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 (λ_{ex} = 383 nm, λ_{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 λ_{ex} = 383±15 nm, λ_{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 λ_{ex} = 383 (±15) nm and λ_{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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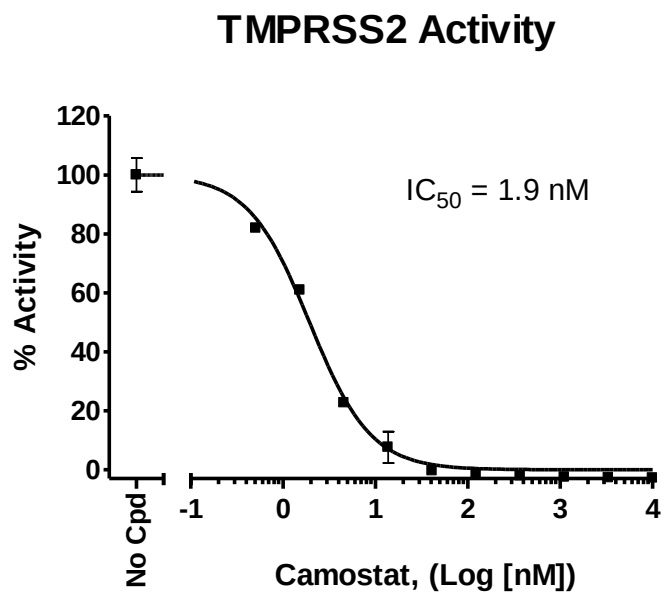
Example of Assay Results:



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