



VERSION 2

APR 17, 2023

OPEN  ACCESS

Protocol Citation: Haim Barr, Noa Lahav 2023. SARS-COV-2 Main Protease (Mpro) Fluorescence Dose Response. **protocols.io** <https://protocols.io/view/sars-cov-2-main-protease-mpro-fluorescence-dose-response-cszqw5w> Version created by [ASAP Discovery](#)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Apr 17, 2023

Last Modified: Apr 17, 2023

PROTOCOL integer ID:
80656

SARS-COV-2 Main Protease (Mpro) Fluorescence Dose Response V.2

Haim Barr^{1,2}, Noa Lahav^{1,2}

¹Weizmann Institute of Science; ²ASAP Drug Discovery Consortium



ASAP Discovery

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

This is a **functional, biochemical assay** used to identify treatments for viral infectious disease that target SARS-COV-2 Main Protease (MPro).

Utilizing a **direct enzyme activity measurement method**, the experiment was performed in a 384-well plate reading the fluorescence intensity. This assay tested the mode of action of inhibition.

It was developed at the Weizmann Institute of Science, as a part of the ASAP Drug Discovery Consortium.

Experiment Concentrations (From Stock to Assay)

A	B	C	D	E

A	B	C	D	E
Reagent	Stock	Concentration when loaded into the Combi (Beginning Concentration)	Final Assay Concentration	Units
SARS Mpro	20000	10	5	nM
SARS Substrate	20000 μ M (intermediate dilution to 100 μ M with DMSO)	750	375	nM

For more information, please check out the "Materials" Section

Assay Buffer Concentrations (From Stock to Assay)

A	B	C	D	E
Reagent	Stock	Concentration when loaded into Combi	Final Assay Concentration	Units
HEPES (pH 7.3)	40	20	20	mM
NaCl	100	50	50	mM
Glycerol	50	10	10	% by volume
TWEEN 20	10	0.01	0.01	% by volume
TCEP	1000	1	1	mM

For more information, please check out the "Materials" Section

GUIDELINES

Plate Information:

Total Assay Volume: 20 μ L

Compounds Top Assay Concentration: 100 μ M

Dilution Factor: 2

Dose Response Points: 12

Number of Replicates: 2

Backfill with DMSO: Yes

MATERIALS

Assay Buffer Reagents (Concentration listed are Stock Solution Concentrations)

1. [M] 40 millimolar (mM)
⊗ HEPES 1M Solution pH 7.3 Fisher Scientific Catalog #AAJ16924K2 (or similar)
2. [M] 100 millimolar (mM)
⊗ Sodium chloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #S9888-25G
(or similar)
3. [M] 50 % volume
⊗ Glycerol Merck MilliporeSigma (Sigma-Aldrich) Catalog #G5516 (or similar)
4. [M] 10 % volume
⊗ TWEEN® 20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9416
(or similar)
5. [M] 1000 millimolar (mM)
⊗ Tris(2-carboxyethyl)phosphine hydrochloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #75259
(TCEP) (or similar)

***Note:** all components are added fresh to the assay buffer before each experiment

Additional Reagents:

[M] 710 micromolar (μM) SARS MPro Enzyme

- The Enzyme original stock was originally [M] 750 micromolar (μM) and was diluted to create aliquots of [M] 20000 nanomolar (nM) using a **storage buffer** (50 mM Tris pH 7.5, 1 mM DTT, 50 mM NaCl, 1 mM EDTA, 50% Glycerol).
- Before an experiment, the 20000 nM aliquots were **diluted with Assay Buffer** to create a [M] 10 nanomolar (nM) solution to be loaded into the Combi.

[M] 20000 micromolar (μM) SARS MPro Substrate

- SARS MPro Substrate Stock ([5-FAM]-AVLQSGFR-[Lys(Dabcyl)-K-amide) was purchased and dissolved in **DMSO** and yielded a concentration of [M] 20000 micromolar (μM)
- Before an experiment, the SARS MPro Substrate Stock had an *intermediate dilution step* with **DMSO** to yield a [M] 100 micromolar (μM) SARS MPro Substrate Solution. Then, before adding the SARS MPro Substrate to the Combi, it was diluted again with **Assay Buffer** to yield a concentration of [M] 750 nanomolar (nM). The final concentration of **SARS MPro Substrate** for the assay was [M] 375 nanomolar (nM)

SAFETY WARNINGS







Please be sure to wear proper Personal Protective Equipment (PPE) while performing this experiment.



BEFORE START INSTRUCTIONS

Note: Inhibitor compounds stock concentration is **20 mM**. Compounds are pre-dispensed into 384 plates and stored at -200°C until use.


Prepare 384 Well Plate



- 1 **PRIME** Multi-Drop Combi Tube Dispensing Cassette with **Assay Buffer** by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.
 - 1.1 **DISPENSE**  10 µL Assay Buffer to Columns **1** and **23** of assay plate
 - **Note:** These will represent the ***inhibitor control columns*** (Contain: Substrate, Assay Buffer, DMSO; **no experimental compounds**)
 - 1.2 **EMPTY** Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). Discard the Assay Buffer discharged from the cassette.
- 2 **PRIME** Multi-Drop Combi Tube Dispensing Cassette with  10 nanomolar (nM) SARS MPro by selecting the **PRIME** button on the Combi Dispenser until the tubes were filled completely.
 - **Note:** Be sure to cycle dispensing several times on a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).
- 2.1 **DISPENSE**  10 µL  10 nanomolar (nM) SARS MPro to Columns **2** through **22** and also Column **24**.

Note:


 -  10 nanomolar (nM) SARS MPro is two times the final concentration for the assay. It is diluted to be a final concentration of  5 nanomolar (nM) SARS MPro .
 - Column 2 and Column 24 are ***neutral control columns*** (Contain: Enzyme, Substrate, DMSO; **no experimental compounds**)
- 2.2 **EMPTY** Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). Discard the

[M] 10 nanomolar (nM) SARS MPro discharged from the cassette.


- 3 **CENTRIFUGE**  15000 rpm, Room temperature, 00:01:00 plate to remove bubbles 1m



- 4 **INCUBATE** plate for  00:15:00 at  Room temperature 15m

- 5 **PRIME** Multi-Drop Combi Tube Dispensing Cassette with **Assay Buffer** by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely. Then, **EMPTY** the Multi-Drop Combi Tube Dispensing Cassette (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied). **Discard the Assay Buffer discharged from the cassette.**

- 6 **PRIME** Multi-Drop Combi Tube Dispensing Cassette with [M] 750 nanomolar (nM) SARS Substrate by selecting the **PRIME** button on the Combi Dispenser until the tubes were filled completely.
 - **Note:** Be sure to cycle dispensing several times on a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).
- 6.1 **DISPENSE**  10 µL [M] 750 nanomolar (nM) SARS Substrate into Columns **1 through 23** (the full plate)

Note:
 - [M] 750 nanomolar (nM) SARS Substrate is two times the final concentration for the assay. It is diluted to be a final concentration of [M] 375 nanomolar (nM) SARS Substrate

- 7 **CENTRIFUGE** plate at  15000 rpm, Room temperature, 00:01:00 in plate centrifuge to remove bubbles 1m

- 8 **INCUBATE** plate at  Room temperature for  00:30:00 30m
⚠ Make sure the plate is protected from light!

Recommended: Clean/Empty the Multi-Drop Combi Reagent Dispenser and Dispensing Cassette during this incubation step

Read Plate Fluorescence

- 9 **READ** and **RECORD** the plate Relative fluorescence units (RFU) via the "**SARS Endpoint protocol**" on the **PERAstar FS Control Software**.

Expected result

Gain 300 should yield ~10,000 RFU in full reaction and ~6,000 RFU in Buffer Control