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SARS-CoV-2 Mpro fluorescence dose response V.4

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We use this protocol and it's working

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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 Assay Concentrations

	A	B	C
	Reagent	Final Assay Concentration	Units
	SARS Mpro	5	nM
	SARS Substrate	375	nM
	HEPES (pH 7.3)	20	mM
	NaCl	50	mM
	Glycerol	10	% by volume
	TWEEN 20	0.01	% by volume
	TCEP	1	mM

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

Guidelines

Plate Information:

Total Assay Volume: 20 µL
Compounds Top Assay Concentration: 100 µM
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

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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

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.

Prepare Reagents

2 **PRIME** Multi-Drop Combi Tube Dispensing Cassette with [M] 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 a clean plate lid (This confirms there are no bubbles in the Dispensing Cassette).

2.1 **DISPENSE** 10 µL [M] 10 nanomolar (nM) SARS MPro to Columns **2** through **22** and also Column **24.**

Note:

- [M] 10 nanomolar (nM) SARS Mpro is two times the final concentration for the assay. It is diluted to be a final concentration of [M] 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 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 24** (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 **PHERAstar FS Control Software**.

Expected result

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