VERSION 2

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OPEN ACCESS

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

Haim Barr[1,2], Noa Lahav[1,2]

[1]The Weizmann Institute of Science; [2]ASAP Discovery

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ABSTRACT

This is a functional, biochemical assay used to identify treatments for viral infectious disease in SARS-COV-2 Main Protease.

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.

GUIDELINES

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

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 is the final concentration within the plate)

- [M] 20 millimolar (mM)
  HEPES Buffer (pH 7.3) **Fisher Scientific Catalog #BP299-1** (or similar)
- [M] 50 millimolar (mM)
  Sodium Chloride **Fisher Scientific Catalog #S271** (or similar)
- [M] 10 % volume Glycerol **Contributed by users Catalog #G5516** (or similar)
- [M] 0.01 % volume
  Tween 20 **Bio-Rad Laboratories Catalog #170-6606-MSDS** (or similar)
- [M] 1 millimolar (mM) TCEP HCl **P212121 Catalog #SV-TCEP** (or similar)

(all components are added fresh to the assay buffer before each experiment)

Additional Reagents

- [M] 5 nanomolar (nM) **SARS Mpro Enzyme***

***Note:** Enzyme original stock was originally [M] 710 micromolar (µM) and was diluted to create smaller aliquots of [M] 20000 nanomolar (nM) (Storage Buffer was : 50 mM Tris pH 7.5, 1 mM DTT, 50 mM NaCl, 1 mM EDTA, 50% Glycerol). The 20000 nM aliquots [M] 20000 micromolar (µM) SARS Substrate were then diluted with **assay buffer** to [M] 10 nanomolar (nM), before each experiment. It was then diluted to be [M] 100 micromolar (µM) with **DMSO**.

- [M] 375 nanomolar (nM) **SARS Substrate***

***Note:** SARS Substrate ([5-FAM]-AVLQSGFR-[Lys(Dabcyl)-K-amide) was dissolved in DMSO with an original concentration of [M] 20000 micromolar (µM). Following an intermediate dilution with DMSO, the SARS Substrate was diluted to a final concentration of 750 nM in assay buffer when it was first loaded into the Multi-Drop Combi Reagent Dispenser. When the entire plate was fully dispensed, the final concentration for SARS Substrate was then 375 nM

SAFETY WARNINGS

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

Prepare 384 Well Plate

15m

1 **PRIME** with **Assay Buffer** by Multi-Drop Combi Tube Dispensing Cassette 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

2 **PRIME** with [M] 10 nanomolar (nM) SARS MPro by Multi-Drop Combi Tube Dispensing Cassette by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing several times on a a clean plate lid

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

- **Note:** Discard the [M] 10 nanomolar (nM) SARS Mpro discharged from the cassette.

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

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

5 **PRIME** with **Assay Buffer** by Multi-Drop Combi Tube Dispensing Cassette by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely. Then, immediately **EMPTY** the Multi-Drop Combi Tube Dispensing Cassette.

6 **PRIME** with [M] 750 nanomolar (nM) SARS Substrate by Multi-Drop Combi Tube Dispensing Cassette by selecting the **PRIME** button on the Combi Dispenser until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing several times on a a clean plate lid

7 **DISPENSE** 10 µL [M] 750 nanomolar (nM) SARS Substrate to 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

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

9 **INCUBATE** plate for 00:30:00 at Room temperature 30m

⚠ Make sure the plate is protected from light

- ***Recommended:*** Clean the Multi-Drop Combi Reagent Dispenser during this incubation step

Read Plate Fluorescence

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

Equipment

PHERAstar FS	NAME
Microplate reader	TYPE
BMG LABTECH	BRAND
0471B0001A	SKU
https://www.bmglabtech.com/en/pherastar-fsx/?utm_term=pherastar%20plate%20reader&utm_campaign=usa.roi.products&utm_source=adwords&utm_medium&gclid=Cj0KCQjw8qmhBhClARIsANAtbodGRjigZtEYwcoMXUtxsLn25xp4gjKra3ZNt9jLh9-FwOoFR_5EUHUaAlkREALw_wcB	LINK

Expected result

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