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We are still developing and optimizing this protocol

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MERS Main Protease (Mpro) Fluorescence Dose Response V.1

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ABSTRACT

This is a functional, biochemical assay used to identify treatments for viral infectious disease in MERS 3C-like 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] 0.1 mg/mL
  BSA-Molecular Biology Grade - 12 mg **New England Biolabs Catalog #B9000S**
- [M] 0.01 % volume
  Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-50ML** (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] 50 nanomolar (nM) **MERS Mpro Enzyme***

***Note:** Enzyme stock was originally [M] 507000 nanomolar (nM) and was diluted with the same assay buffer used in the experiment before conducting each experiment

- [M] 550 nanomolar (nM) **MERS Substrate***

***Note:** MERS Substrate (5-FAM)-GVLQSGLV-K(Dabcyl)-K-NH2 Stock was purchased from Peptide 2.0 and dissolved in DMSO with an original concentration of [M] 750000 nanomolar (nM) however it was diluted with the same assay buffer used in the experiment before conducting each experiment

SAFETY WARNINGS

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

ETHICS STATEMENT

N/A

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 (by selecting the **EMPTY** button on the Combi Dispenser until the tubes of the cassette are emptied).

- Discard Assay Buffer discharged from the cassette.

2 **PRIME** with [M] 100 nanomolar (nM) MERS MPro by Multi-Drop Combi Tube Dispensing Cassette 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] 100 nanomolar (nM) MERS MPro to Columns **2 through 22** and Column **24**

Note:

- [M] 100 nanomolar (nM) MERS MPro is two times the final concentration for the assay. It is diluted to be a final concentration of [M] 50 nanomolar (nM) MERS 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.)

- Discard the [M] 100 nanomolar (nM) MERS 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** with **Assay Buffer** by Multi-Drop Combi Tube Dispensing Cassette by selecting the **PRIME** button on the Combi Dispenser until the tubes were filled completely. Then, **EMPTY** the Multi-Drop Combi Tube Dispensing Cassette.

6 **PRIME** with [M] 1100 nanomolar (nM) MERS Substrate by Multi-Drop Combi Tube Dispensing Cassette 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).

7 **DISPENSE** 10 µL 1100nM MERS Substrate into Columns 1 through 23 (the full plate)

Note:

- [M] 1100 nanomolar (nM) MERS Substrate is two times the final concentration for the assay. It is diluted to be a final concentration of [M] 50 nanomolar (nM) MERS Substrate

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

9 **INCUBATE** plate for 01:00:00 at Room temperature 1h

⚠ **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 **"MERS Protocol"** on the **PHERAstar FS Control Software.**

- Software is a standard Flourescence Assay set for Optimal excitation wavelength 485 nm, emission wavelength 528 nm, and a Gain of 300.

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