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**Protocol status:** In development
We are still developing and optimizing this protocol

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# MERS Main Protease (Mpro) Fluorescence Dose Response V.3

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ASAP Discovery

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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.

## 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