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MERS-CoV Mpro fluorescence dose response for antiviral testing V.5

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

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Disclaimer

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Abstract

This is a functional, biochemical assay used to identify treatments for viral infectious disease in MERS-CoV 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.
This assay can run as dose response or single point as described in the guidelines tab.

Final Experiment Concentrations

	A	B	C
	Reagent	**Concentration**	**Units**
	MERS Mpro	50	nM
	MERS Substrate peptide	550	nM
	HEPES pH=7.3	20	mM
	NaCl	50	mM
	BSA	0.1	mg/ml
	Triton X-100	0.01	% (v/v)
	TCEP	1	mM

For more information, please see the Materials section.

Guidelines

Compound Plate Design for Dose Response:

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

Compounds Plate Design for 2-Point Assay:

Total Assay Volume: 20 µL
Compounds Assay Concentration: 100 µM and 50 µM
Dilution Factor: 2
Dose Response Points: 2
Number of Replicates: 2
Backfill with DMSO: Yes

Materials

Assay Buffer Reagents (Concentration listed are the stock concentrations)

- [M] 40 millimolar (mM) HEPES Buffer (pH 7.3) **Fisher Scientific Catalog #**BP299-1 (or similar)
- [M] 100 millimolar (mM) Sodium Chloride **Fisher Scientific Catalog #**S271 (or similar)
- [M] 10 mg/mL BSA-Molecular Biology Grade - 12 mg **New England Biolabs Catalog #**B9000S (or similar)
- [M] 10 % volume Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787-50ML (or similar)
- [M] 1000 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] 507000 nanomolar (nM) **MERS Mpro Enzyme***

***Note:** Stock solutions were diluted with fresh assay buffer to create a [M] 100 nanomolar (nM) solution before each experiment

- [M] 750000 nanomolar (nM) **MERS Substrate***

***Note:** MERS Substrate (5-FAM)-GVLQSGLV-K(Dabcyl)-K-NH2 Stock was purchased from Peptide 2.0, dissolved in DMSO to [M] 750000 nanomolar (nM), aliquoted and stored at -80 °C. Before each experiment, stock was diluted with assay buffer to yield a concentration of [M] 1100 nanomolar (nM).

Protocol materials

Sodium Chloride **Fisher Scientific Catalog #**S271

BSA-Molecular Biology Grade - 12 mg **New England Biolabs Catalog #**B9000S

TCEP HCl **P212121 Catalog #**SV-TCEP

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787-50ML

HEPES Buffer (pH 7.3) **Fisher Scientific Catalog #**BP299-1

Middle East respiratory syndrome coronavirus (MERS-CoV) Mpro strain HCoV-EMC 3CL protease domain **addgene Catalog #**228646

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 -20 C until use.

Protein expression and purification

1 We used the following plasmid in the protein expression and purification protocol.

Middle East respiratory syndrome coronavirus (MERS-CoV) Mpro strain HCoV-EMC 3CL protease domain **addgene Catalog #**228646

Protocol

NAME

MERS-CoV Mpro large scale purification protocol

CREATED BY

Korvus Wang

PREVIEW

Prepare Reagents

2 **PREPARE** all of the reagents/buffers required for this experiment.

	A	B	C	D	E
	Reagent	**Stock Concentration**	**Concentration Loaded into dispenser**	**Final Concentration in plate**	**Units**
	MERS Mpro Enzyme stock	507000	100	50	nM
	MERS Substrate	750000	1100	550	nM
	Assay Buffer				
	HEPES (pH 7.3)	40	20	20	mM
	Sodium Chloride	100	50	50	mM
	BSA	10	0.1	0.1	mg/mL
	Triton X-100	10	0.01	0.01	% by volume
	TCEP	1000	1	1	mM

For more information, please see the Materials sec

Prepare 384 Well Plate

15m

3 **PRIME** the dispenser with **Assay Buffer** by selecting the **PRIME** button until the tubes are filled completely.

3.1 **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**)

3.2 **EMPTY** the dispenser tubes.

- Discard Assay Buffer discharged from the dispenser tubes.

4 **PRIME** the dispenser with [M] 100 nanomolar (nM) MERS MPro Enzyme by selecting the **PRIME** button until the tubes are filled completely.

4.1 **DISPENSE** 10 µL [M] 100 nanomolar (nM) MERS MPro Enzyme to Columns **2 through 22** and Column **24**

Note:

- Be sure to cycle dispensing several times on an empty plate to detect and avoid dispensing issues.
- [M] 100 nanomolar (nM) MERS MPro Enzyme is two times the final concentration for the assay. It is diluted in the plate to a final concentration of [M] 50 nanomolar (nM) MERS MPro Enzyme .
- Column 2 and 24 are ***neutral control columns*** (Contain: Enzyme, Substrate, DMSO, no compounds)

4.2 **EMPTY** the dispenser tubes .

- Discard the [M] 100 nanomolar (nM) MERS MPro enzyme discharged from the tubes.

5 **CENTRIFUGE** plate 1500 rpm, Room temperature, 00:01:00 to remove bubbles 1m

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

During Incubation: wash and prepare the dispenser to the next step

7 **PRIME** the dispenser with [M] 1100 nanomolar (nM) MERS Substrate by selecting the **PRIME** button until the tubes are filled completely.

- **Note:** Be sure to cycle dispensing on an empty plate to detect and avoid dispensing issues.

8 **DISPENSE** 10 µL 1100nM MERS Substrate into Columns **1 - 24** (the full plate)

Note:

- [M] 1100 nanomolar (nM) MERS Substrate is two times the final concentration for the assay. It is diluted in the plate to a final concentration of [M] 550 nanomolar (nM) MERS Substrate

9 **CENTRIFUGE** plate 1500 rpm, Room temperature, 00:01:00 in plate centrifuge to remove bubbles 1m

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

⚠ **Make sure the plate is protected from light!**

Recommended: Clean the dispenser during this incubation step

Read Plate Fluorescence

11 **READ** and **RECORD** the plate Relative fluorescence units (RFU) via the **"MERS Protocol"** on the **PHERAstar FS Control Software.**

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

Equipment

PHERAstar FS

Microplate reader

BMG LABTECH

0471B0001A

https://www.bmglabtech.com/en/pherastar-fsx/?utm_term=pherastar%20plate%20reader&utm_campaign=usa.roi.products&utm_source
FwOoFR_5EUHUaAIkREALw_wcB

Expected result

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

Experimental Design

12 Figure 1: Graphical depiction of assay principal and its use in screening campaign