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We use this protocol and it's working

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

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 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.

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

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 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:** The original MERS Mpro stock enzyme had a concentration of [M] 507000 nanomolar (nM) but when a new stock solution was made/delivered on 2023-02-21the new stock had a concentration of [M] 478000 nanomolar (nM) Both 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 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 to yield a concentration of [M] 1100 nanomolar (nM)

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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

Prepare Reagents

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

	A	B	C	D	E
	Reagent	Stock Concentration	Concentration Loaded into Combi	Final Concentration	Units
	MERS Mpro Enzyme (original stock)	507000	100	50	nM
	20230221 MERS Mpro Enzyme	478000	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

2 **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.

2.1 **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**)

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 Assay Buffer discharged from the cassette.

3 **PRIME** with [M] 100 nanomolar (nM) MERS MPro Enzyme 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).

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

Note:

- [M] 100 nanomolar (nM) MERS MPro Enzyme is two times the final concentration for the assay. It is diluted to be a final concentration of [M] 50 nanomolar (nM) MERS MPro Enzyme.
- Column 2 and Column 24 are ***neutral control columns*** (Contain: Enzyme, Substrate, DMSO, no compounds)

3.2 **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 enzyme discharged from the cassette.

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

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

⚠ Make sure the plate is protected from light!

6 **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.

7 **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).

8 **DISPENSE** 10 µL 1100nM MERS Substrate into Columns **1 through 24** (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

9 **CENTRIFUGE** 15000 rpm, Room temperature, 00:01:00 plate 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 Multi-Drop Combi Reagent 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 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