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Fluorescence assay for MERS-CoV Mpro protease activity measurement for screening against antiviral molecules V.2

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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 protocol details the fluorescence assay for MERS-CoV Mpro protease activity measurement. This method is intended to measure the activity of viral proteases by using a specific labelled peptide that allows the detection of the cleaved product. The substrate contains the cleavage-sequence specific to the tested protease and is labeled in C-terminal by the fluorophore Edans (ex 336 nm; em: 455 nm) and in N-ternimal by the quencher Dabcyl (ex 472 nm). In the case of a non-cleaved substrate, the proximity of Dabcyl to Edans prevents the emission and the detection of the fluorescence at 455 nm. The cleavage of the peptide by the protease allows Edans' fluorescence emission and detection. In this current version we added the protein production protocol and the plasmid used for this assay. In this new version, we added the protocol for protein expresssion and purification and the Addgene id.

Attachments

DOCX

nyiwb5id7.docx

203KB

Materials

Reagents

- **Assay buffer:** [M] 25 millimolar (mM) HEPES pH 7.5, [M] 150 millimolar (mM) NaCl, [M] 10 millimolar (mM) glycerol, [M] 7 millimolar (mM) DTT, [M] 5 millimolar (mM) EDTA, [M] 1 millimolar (mM) TCEP (optional) and [M] 0.05 % volume Tween-20 .
- **Incubation**: 01:00:00 at Room temperature .
- **MERS-CoV Mpro**: Protein stocks were stored at -80 °C and used as 2x solution ([M] 10 micromolar (µM) , [M] 5 micromolar (µM) final assay concentration) in assay buffer.
- **Substrate**: Edans-GVLQSGLV-LysDabcyl-K (LifeTein, USA) prepared as a stock solution at [M] 10 millimolar (mM) in DMSO and used at 2x solution ([M] 20 micromolar (µM) , [M] 10 micromolar (µM) final concentration) in assay buffer.
- **Positive control**: GC376 (Pubchem CID 71481119), [M] 50 micromolar (µM) top final assay concentration.
- **Plates**: Greiner, 384-well plate, black, small volume, non-binding #7840900
- **Liquid handler**: Echo® acoustic liquid handler (Beckman Coulter, USA).
- **Plate reade**r: Pherastar FS, BMG Labtech (Germany), 350-460 FI optic module, the plate is read every 30 s for 2 hours and shacked during 5 s before the first reading.

Protocol materials

MERS-CoV Mpro **addgene Catalog** #228646

Protein production

2d

1 The protein used in this assay was expressed and purified following the protocol below with plasmid MERS-CoV Mpro **addgene Catalog** #228646

Protocol

NAME

MERS-CoV Mpro large scale purification protocol

CREATED BY

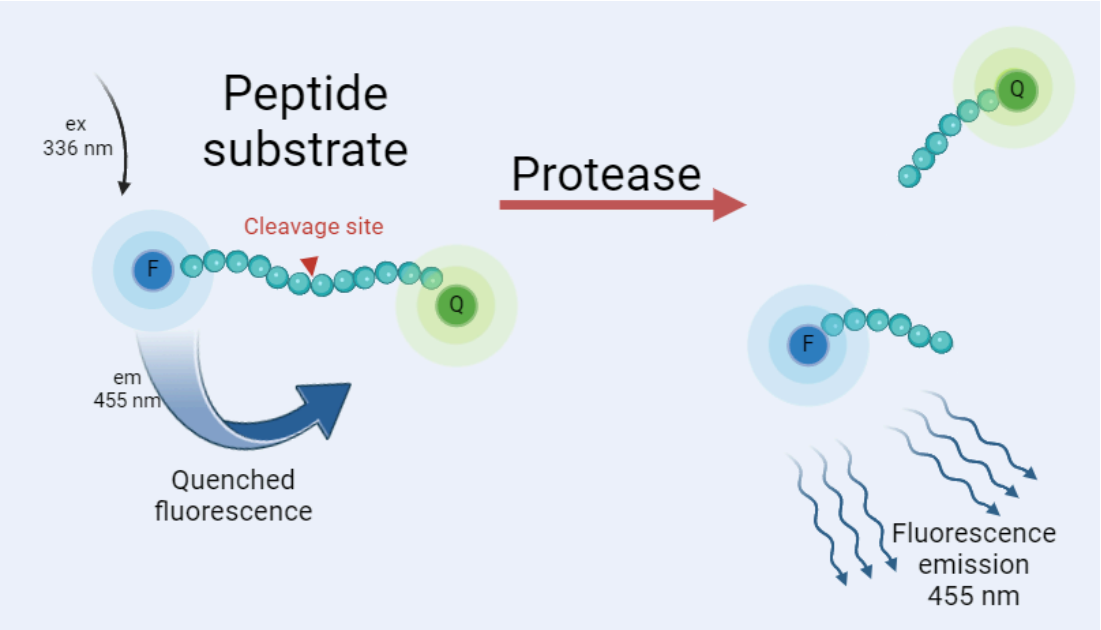
korvus.wang

Preview

MERS-CoV Mpro Assay

3h

2 This method is intended to measure the activity of viral proteases by using a specific labelled-peptide that allows the detection of the cleaved product. The substrate contains the cleavage-sequence specific to the tested protease and is labeled in C-terminal by the fluorophore Edans (ex 336 nm; em: 455 nm) and in N-ternimal by the quencher Dabcyl (ex 472 nm). In the case of a non-cleaved substrate, the proximity of Dabcyl to Edans prevents the emission and the detection of the fluorescence at 455 nm. The cleavage of the peptide by the protease allows Edans' fluorescence emission and detection.



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Reagents and equipment

3h 0m 35s

3 3h 0m 35s

Assay buffer: [M] 25 millimolar (mM) HEPES pH 7.5, [M] 150 millimolar (mM) NaCl, [M] 10 millimolar (mM) glycerol, [M] 7 millimolar (mM) DTT, [M] 5 millimolar (mM) EDTA, [M] 1 millimolar (mM) TCEP (optional) and [M] 0.05 % volume Tween-20 .

Incubation: 01:00:00 at room temperature.

MERS-MPro: protein stocks were stored at -80C and used as [M] 2 x solution ([M] 10 micromolar (µM) , [M] 5 micromolar (µM) final assay concentration) in assay buffer.

Positive control: GC376 (Pubchem CID **71481119**), [M] 25 micromolar (µM) top final assay concentration.

Substrate: Edans-GVLQSGLV-LysDabcyl-K (LifeTein, USA) prepared as a stock solution at 10 mM in DMSO and used at 2x solution ([M] 20 micromolar (µM) , [M] 10 micromolar (µM) final concentration assay concentration) in assay buffer.

Liquid handler: Echo® acoustic liquid handler (Beckman Coulter, USA).

Plate reader: Pherastar FS, BMG Labtech (Germany), 350-460 FI optic module, the plate is read every 00:00:30 for 02:00:00 and shacked 00:00:05 before each reading.

MERS-MPro IC50 Measurement

3h 0m 30s

4 Add 10 µL of 2x protein [M] 10 micromolar (µM) solution to each well containing the compounds to be tested previously dispensed onto the plate.

5 Incubate the mix for 01:00:00 at Room temperature to initiate the enzymatic reaction by the addition of 10 µL of 2x ([M] 20 micromolar (µM)) substrate solution using the plate reader injector.

1h

6 Read the fluorescence intensity at 350/460 nm every 00:00:30 for 02:00:00 in kinetic mode, which includes a shaking step of the plate before each measurement.

2h 0m 30s

7 Calculate the IC50 by plotting the initial velocity against various concentrations of tested inhibitors by using a four-parameter dose–response curve in Prism (v8.0) software.

8 Calculate the mean (µ) and the standard deviation (σ) of fluorescence intensity and then calculate the signal-to-background ratio and the Z' or Z-factor. (s: signal; c: control).

$$\text{Estimated Z-factor} = 1 - \frac{3(\hat{\sigma}_s + \hat{\sigma}_c)}{|\hat{\mu}_s - \hat{\mu}_c|}$$

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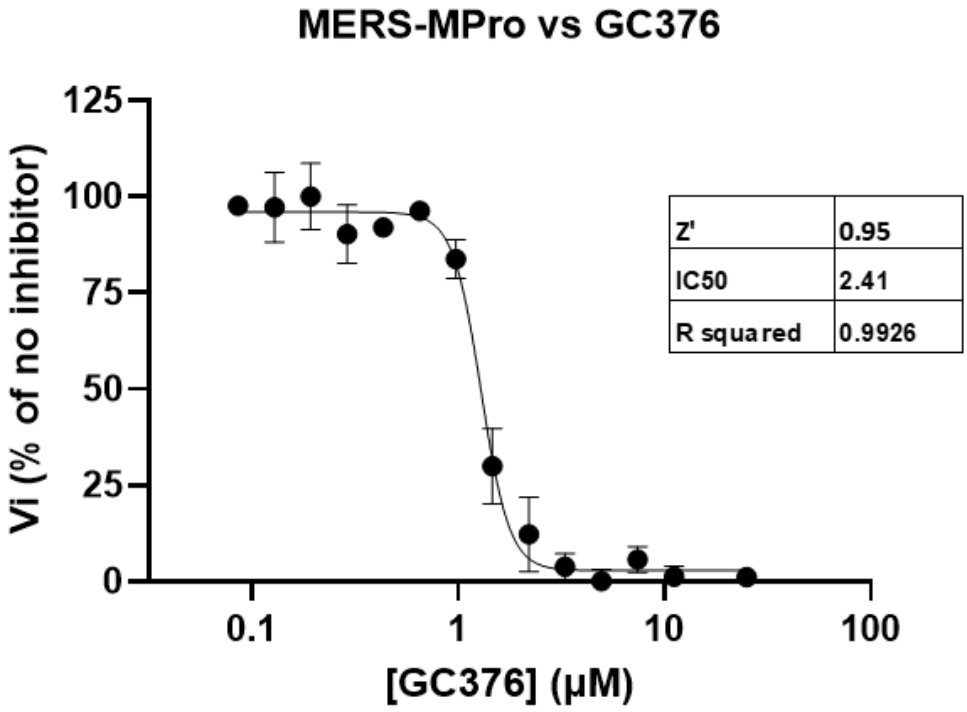
MERS-MPro vs GC376
Vi (% of no inhibitor)
125
100
75
50
25
0
0.1
1
10
100
[GC376] (µM)
Z'
0.95
IC50
2.41
R squared
0.9926

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