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Fluorescence assay of EV-D68 3C 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 Enterovirus D68 (EV-D68) 3C 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 new version, we changed the buffer to be able to reduce the concentration of enzyme used. Also, we added the correct affiliation for the authors, protocol for protein purification and the Addgene ID for the corresponding plasmid used for protein production.

Attachments

DOCX

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203KB

Materials

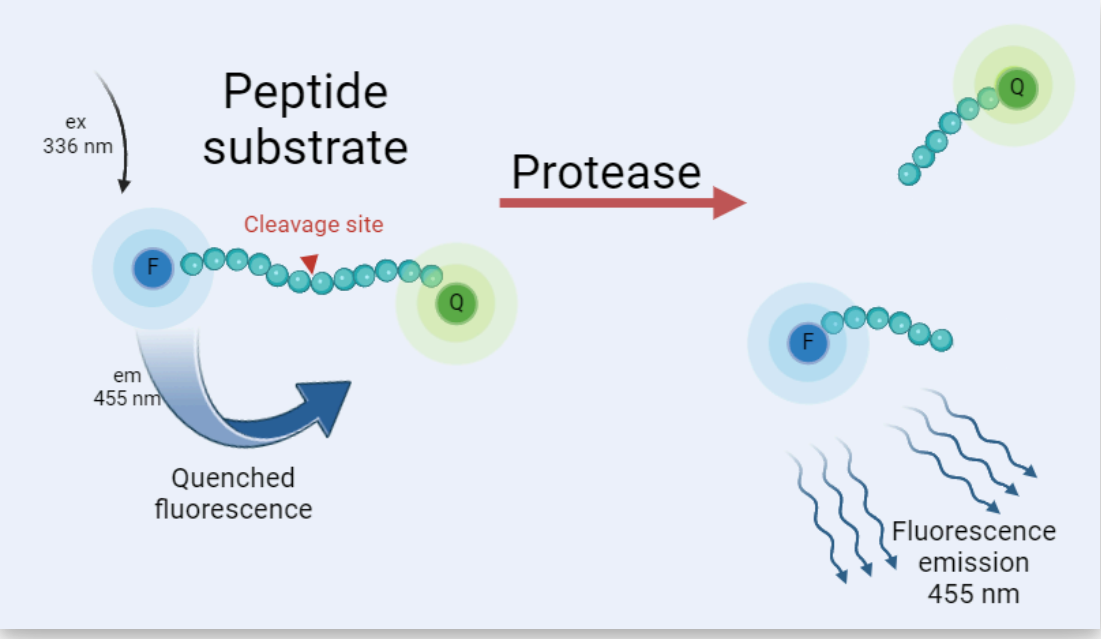
Reagents

- **Assay buffer**: [M] 25 millimolar (mM) HEPES pH 7.5, [M] 150 millimolar (mM) NaCl, [M] 10 millimolar (mM) glycerol and [M] 7 millimolar (mM) DTT, [M] 5 millimolar (mM) EDTA, [M] 0.05 % volume Tween-20.
- **Incubation**: 01:00:00 at Room temperature.
- **EV-D68 3C protease**: Protein stocks were stored at -80 °C and used as 2x solution ([M] 1 micromolar (µM) , [M] 0.5 micromolar (µM) final assay concentration) in assay buffer.
- **Substrate**: Dabcyl-KEALFQGPPQFE-Edans (LifeTein, USA) prepared as a stock solution at [M] 10 millimolar (mM) in DMSO and used at 2x solution ([M] 20 millimolar (mM) , [M] 10 millimolar (mM) final concentration) in assay buffer.
- **Positive control**: GC376 (Pubchem CID 71481119), [M] 5 micromolar (µM) top final assay concentration.
- **Plates**: Small-volume 384-well plate, black, Greiner cat# 784900.
- **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.

EV-D68 3C protease assay

3h

1 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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Protein expression and purification

2 The enzyme used in this assay was expressed and purified following the protocol bellow.

Protocol

NAME

Enterovirus D68 3C protease small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

3 Another construct that we used is the one bellow:

Protocol

NAME

Small-scale Expression and Purification Protocol for His-SUMO Tagged Enterovirus D68 Strain STL 2014 12 3C Protease in E. coli

CREATED BY

korvus.wang

Preview

:

Reagents and equipment

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

Incubation: 01:00:00 at Room temperature .

EV-D68 3C: protein stocks were stored at -80C and used as 2x solution ([M] 2 micromolar (µM) , [M] 1 micromolar (µM) final assay concentration) in assay buffer.

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

Positive control: GC376 (Pubchem CID 71481119), 5 µM top final assay concentration.
Plates: Greiner, 384-well plate, black, small volume, non-binding #7840900
Plate reader: Pherastar FS, BMG Labtech (Germany), 350-460 FI optic module, the plate is read every 30 seconds for 2 hours and shacked 5 s before each reading.

EV-D68 3C Pro IC50 Measurement

3h

5 Add 10 µL of 2x protein [M] 1 micromolar (µM) solution to each well containing the compounds to be tested previously dispensed onto the plate.

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

1h

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

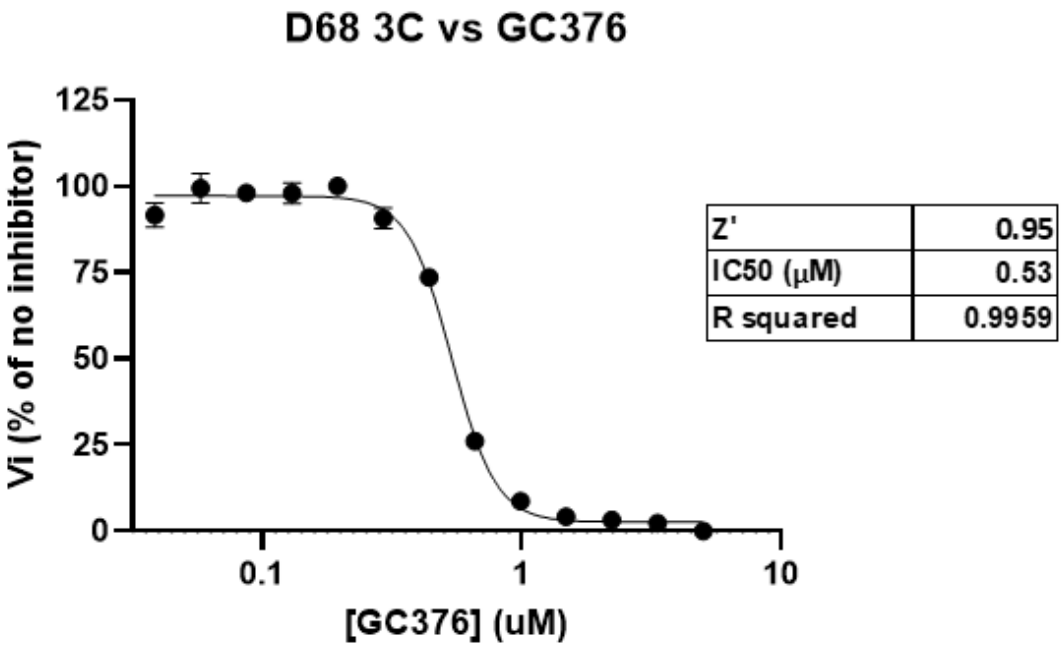
2h

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

9 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).

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

10


D68 3C vs GC376
Vi (% of no inhibitor)
125
100
75
50
25
0
0.1
1
10
[GC376] (uM)
Z' 0.95
IC50 (μM) 0.53
R squared 0.9959

Asset URL: