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Fluorescence assay for Enterovirus A71 3C protease activity measurement for screening against antiviral molecules V.2

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Charline Giroud[1,2,3], Oleg Fedorov[1,2,3]

[1]Center for Medicines Discovery; [2]University of Oxford; [3]ASAP Discovery Consortium

ASAP Discovery

Charline Giroud
Center for Medicines Discovery

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

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Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract

This protocol details the fluorescence assay for Enterovirus A71 (EV-A71) 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.

Attachments

DOCX

nyiwb5id7.docx

203KB

Materials

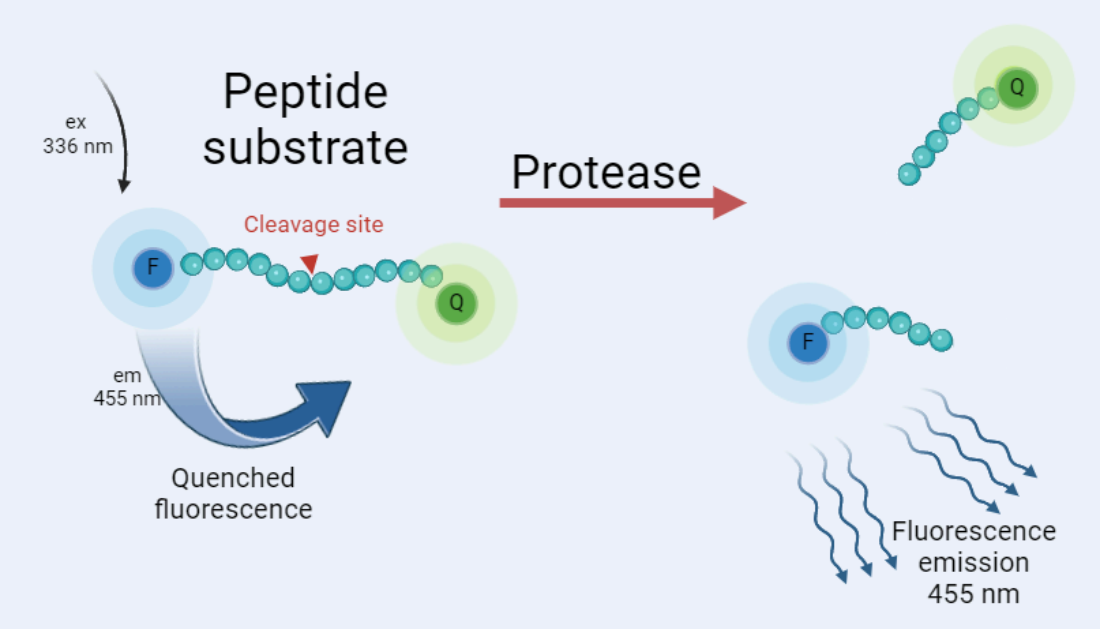
Reagents

- **Assay buffer**:
- **Incubation**: 01:00:00 at Room temperature .
- **EVA71 3C**: 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**: Dabcyl-KEALFQGPPQFE-Edans (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**: 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-A71 3C Pro 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.



Asset URL:

Protein expression and purification

2 Protocol used for protein expression and purification is the one bellow.

Protocol

NAME

Enterovirus A71 3C protease small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

EV-A71 3C Pro IC50 Measurement

3h

3 **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.

3h 0m 35s

Incubation: 01:00:00 at Room temperature .

EV-A71 3C: protein stocks were stored at -80C and used as 2x solution ([M] 10 micromolar (µM) , [M] 5 micromolar (µM) final assay concentration) in assay buffer.

Positive control: GC376 (Pubchem CID 71481119), 25 µM top final assay concentration.

Substrate: Dabcyl-KEALFQGPPQFE-Edans (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.

- **Plates**: Small-volume 384-well plate, non-binding, black, Greiner cat# 784900.

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.

4 10 µL of 2x protein solution were added 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 and initiate the enzymatic reaction by adding of 10 µL of 2x ([M] 20 micromolar (µM)) substrate solution using the plate reader injector. 1h

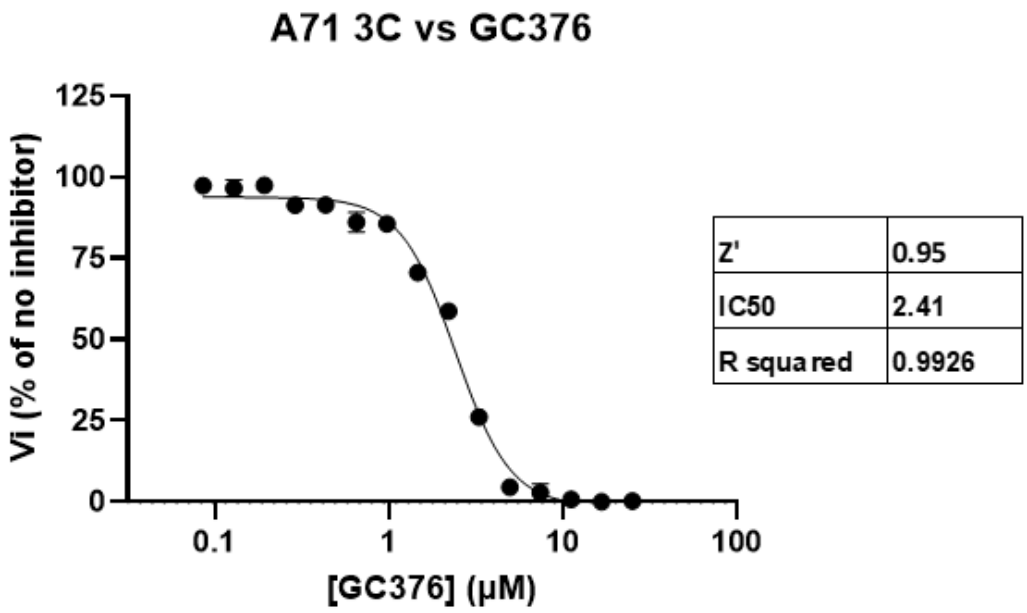
6 Read the fluorescence intensity at 350/460 nm every 30 seconds for 02:00:00 in kinetic mode, which includes a shaking step of the plate before each measurement. 2h

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).

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

9


A71 3C 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

Asset URL: