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Fluorescence assay for Enterovirus coxsackievirus A16 2A protease activity measurement V.1

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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 coxsackievirus A16 (CVA16) 2A 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

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Materials

Reagents

- **Assay buffer**:

	A	B
	Tris pH 7.0	50 mM
	NaCl	150 mM
	Glycerol	10%
	DTT (optional)	0.5 mM

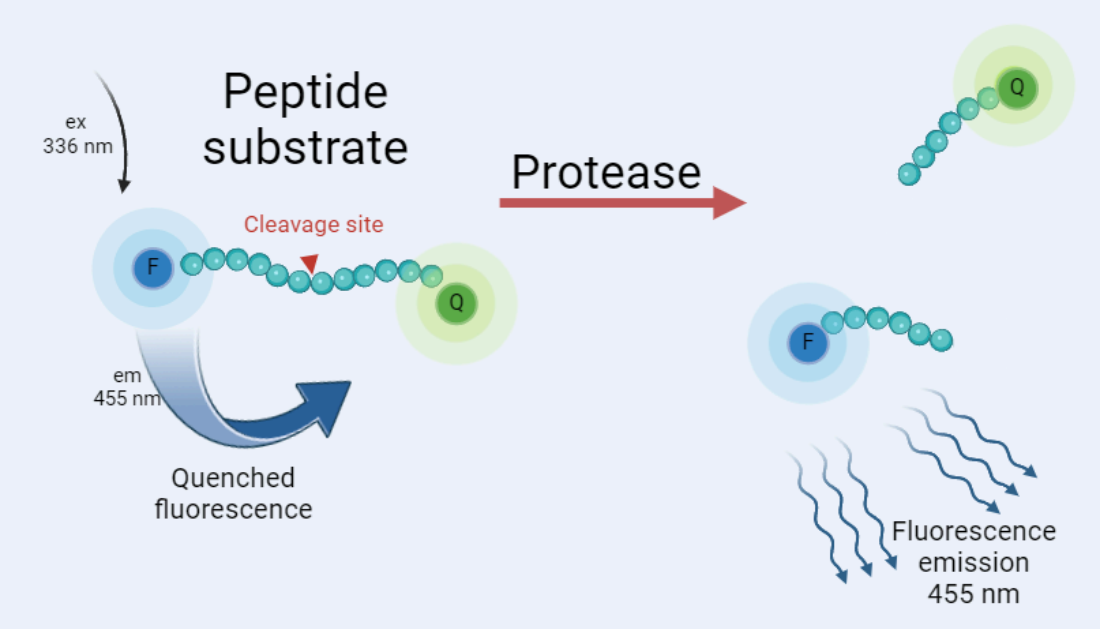
- **Incubation**: 01:00:00 at Room temperature .
- **EV-D68 3C**: Protein stocks were stored at -80 °C and used as 2x solution ([M] 1 micromolar (µM) , [M] 0.5 micromolar (µM) final assay concentration) in assay buffer.
- **Substrate**: Dabcyl-KEALFQGPPQFE-Edans (LifeTein, USA) prepared as a stock solution at [M] 5 millimolar (mM) in DMSO and used at 2x solution ([M] 40 micromolar (µM) , [M] 20 micromolar (µM) final concentration assay concentration) in assay buffer.
- **Positive control**: GC376 (Pubchem CID 71481119), [M] 50 micromolar (µM) top final assay concentration.
- **Plates**: ProxiPlate-384 Plus, white, Greiner cat# 6008280.
- **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.

Troubleshooting

CVA16 2A 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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Reagents and equipment

2 **Assay buffer:** [M] 50 millimolar (mM) Tris pH 7.0, [M] 150 millimolar (mM) NaCl, [M] 10 % (v/v) glycerol and [M] 1 millimolar (mM) TCEP (optional). 3h 0m 35s

Incubation: 01:00:00 at room temperature.

CVA16 2A protease: protein stocks were stored at -80C and used as [M] 2 Mass Percent solution ([M] 5 micromolar (µM) , [M] 2.5 micromolar (µM) final assay concentration) in assay buffer.

Positive control: Telaprevir (Pubchem CID 3010818), [M] 50 micromolar (µM) top final assay concentration.
Substrate: Dabcyl-TAITTLGKFGQE-Edans (LifeTein, USA) prepared as a stock solution at 10 mM in DMSO and used at 2x solution ([M] 20 micromolar (µM) , [M] 2.5 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.

CVA16 2A protease IC50 Measurement

3h

3 Add 50 µL of 2x protein [M] 0.5 micromolar (µM) solution to each well containing the compounds to be tested previously dispensed onto the plate.

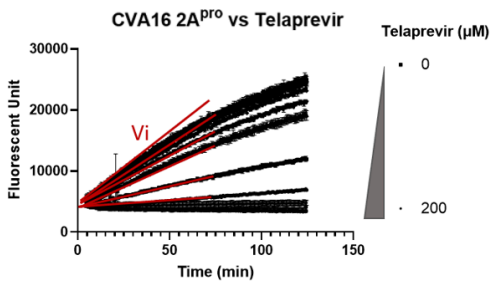
4 Incubate the mix for 01:00:00 at Room temperature and initiate the enzymatic reaction by the addition of 50 µL of 2x ([M] 10 micromolar (µM)) substrate solution using the plate reader injector.

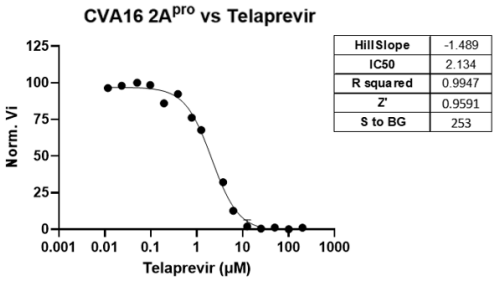
1h

5 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

6 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.





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7 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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