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ab211089
HRV 3C Protease Inhibitor Screening Kit (Colorimetric)

For the rapid, sensitive and accurate screening of potential HRV 3C Protease inhibitors.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

HRV 3C Protease Inhibitor Screening Kit (Colorimetric) (ab211089) provides a rapid, simple, sensitive and reliable test suitable for high-throughput screening of HRV 3C Protease inhibitors. The assay is based on the ability of a 3C Protease (derived from a HRV rhinovirus-14) to cleave a chromogenic peptide substrate to release a chromophore (*p*NA) which can be easily quantified using a colorimetric microplate reader. In the presence of a HRV 3C Protease-specific inhibitor, the cleavage of the substrate is reduced/abolished resulting in decrease or total loss of the absorbance.

This simple and high-throughput adaptable assay kit can be used to screen/study/characterize potential inhibitors of HRV 3C Protease.

HRV 3C substrate-*p*NA → (HRV 3C protease) → Cleaved substrate + *p*NA (absorbance @ OD 405 nm)

HRV 3C substrate-*p*NA → (HRV 3C protease + Inhibitor) → Decrease in absorbance/ No absorbance

Human rhinovirus (HRV) infections are the most frequent causative agents of common cold and various other upper respiratory tract infections. Rhinoviruses are members of the picornavirus family, which have a positive-sense, single-stranded RNA genome that is translated into a single polyprotein precursor. In the case of HRVs, the viral polyprotein is mainly processed by the proteases (2A and 3C) to generate functional proteins and enzymes.

Human rhinovirus 3C protease (EC: 3.4.22.28) is a cysteine protease that recognizes the cleavage site Leu-Glu-Val-Leu-Phe-Gln*Gly-Pro.

2. Protocol Summary

Screening compound & controls preparation

Enzyme and substrate solution preparation

Add enzyme solution to wells.
Incubate for 15 minutes at RT

Add Substrate Solution to wells

Measure absorbance at OD 405 nm in kinetic mode
for 1 – 2 hours at room temperature

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ **Note:** Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (Before prep)	Storage temperature (After prep)
HRV 3C Protease Assay Buffer	25 mL	-20°C	-20°C
HRV 3C Protease	2 x 100 µL	-20°C or -80°C	-80°C
HRV 3C Protease Substrate	500 µL	-20°C or -80°C	-80°C
Protease Inhibitor I	20 µL	-20°C	-20°C

PLEASE NOTE: Protease Inhibitor I was previously labelled as HRV 3C Protease inhibitor. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 405 nm
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, controls and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 HRV 3C Protease Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 HRV 3C Protease (2 x 100 µL):

Ready to use as supplied. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -80°C for long term. Avoid repeated freeze/thaw. Each vial contains enough enzyme solution for 50 assays.

9.3 HRV 3C Protease Substrate (500 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -80°C.

9.4 Protease Inhibitor I (20 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot Protease Inhibitor I so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. Sample Preparation

General sample information:

– Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

10.1.1 Dissolve test inhibitors into proper solvent.

10.1.2 Dilute to 10X the desired test concentration with HRV 3C Assay Buffer before use.

Δ **Note**: We suggest using different concentrations of test compounds if effective concentration is unknown.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

Δ Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well with the same final concentration of the solvent as in the inhibitor sample as solvent control.

11.1 Set up Reaction wells:

- Screening samples compound wells (S) = 10 µL test compounds.
- Inhibitor Control wells (IC) = 1 µL Protease Inhibitor I + 9 µL Assay Buffer.
- Enzyme Control wells (EC) = 10 µL Assay Buffer.
- OPTIONAL: Solvent control (SC) = 10 µL solvent.

11.2 Prepare HRV 3C Protease Enzyme Solution:

11.2.1 Prepare 50 µL of enzyme solution for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	HRV 3C Protease Enzyme Mix (µL)
HRV 3C Protease Assay Buffer	48
HRV 3C Protease	2

11.2.2 Mix well and add 50 µL/well into each well.
11.2.3 Incubate plate at room temperature for 15 minutes.

11.3 HRV 3C Substrate Preparation:

11.3.1 Prepare 40 µL of Substrate Solution Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	HRV 3C Protease Substrate Mix (µL)
HRV 3C Protease Assay Buffer	35
HRV 3C Protease Substrate	5

11.3.2 Add 40 µL of Substrate Mix into each well. Mix well.

11.4 Measurement:

11.4.1 Measure immediately absorbance (OD = 405 nm) on a microplate reader in a kinetic mode for 1-2 hours at room temperature.

11.4.2 Choose two time points (T1 and T2) in the linear range of the curve and obtain the corresponding values for the absorbance (A1 and A2).

12. Calculations

– Use only the linear rate for calculation.

12.1 Plot readings for each sample test compound (S), inhibitor control (IC) and enzyme control (EC).

12.2 Draw the line of the best fit to construct the curve (most plate reader software or Excel can do this step). Calculate the trend line equation (use the equation that provides the most accurate fit).

12.3 Choose two points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2).

12.4 Calculate Slope (ΔOD/ΔT) for all samples (S), Enzyme Control (EC) and Inhibitor control (IC), if desired, as follows:

$$\Delta OD/\Delta T = (OD2 - ODU1)/ (T2 - T1)$$

12.5 Average the slope for each duplicate reading.

12.6 Calculate the % Relative Inhibitions as follows:

$$\%\ Relative\ Inhibition = \frac{Slope\ of\ EC - Slope\ of\ S}{Slope\ of\ EC}\ x\ 100$$

Δ Note: Irreversible inhibitors that inhibit HRV 3 C Protease activity completely at the tested concentration will have ΔA = 0 and thus % Relative Inhibition will be 100%.

Δ Note: If RFU of SC < RFU of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent if possible.

If RFU of S < RFU of EC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. Typical Data

Data provided **for demonstration purposes only**.

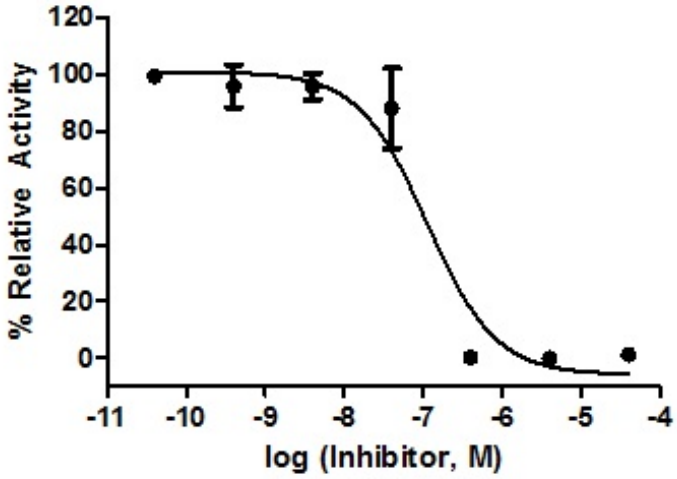

Figure 1. Typical inhibition curve of HRV 3C protease activity by Protease Inhibitor I (IC_{50} = 0.12 µM). Assay was performed following the kit protocol.

14. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot if necessary; get equipment ready.
- Prepare test compounds in suitable solvents; dilute if appropriate.
- Prepare Enzyme Solution (50 µL/well) by adding 2 µL of HRV 3C protease to 48 µL of Assay Buffer. Prepare a mix for all wells.
- Prepare Substrate mix (40 µL/well) by adding 5 µL of HRV 3C Substrate to 35 µL of Assay Buffer. Prepare a mix for all wells.
- Set up plate as follows:

Component	Sample (S) (µL)	Solvent control (SC) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Test Compound	10	0	0	0
Protease Inhibitor I control	0	0	0	1
Solvent test compound	0	10	0	0
Assay Buffer	0	0	10	9
HRV 3C protease Enzyme Solution	50	50	50	50
Incubate 15 minutes at RT				
Add 40 µL HRV 3C Substrate Mix				

- Measure plate in a colorimetric plate reader at OD= 405 nm in kinetic mode for 1 – 2 hours at room temperature.

15. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Assay with erratic readings	Pipetting errors	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
No fluorescence above background in inhibitor wells	Inhibitor concentration is too high	Reduce concentration of inhibitor and re-do assay
No inhibition seen in test compound wells	Inhibitor concentration is not high enough	Increase concentration of inhibitor and re-do assay
	Compound is not an inhibitor	Use another compound for your test

16. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)