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# HRV 3C Protease Inhibitor Screening Kit (Colorimetric)



**(Catalog # K215-100; 100 assays, Store kit at -20°C)**

## I. Introduction:

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. BioVision's HRV 3C Protease Inhibitor Screening Kit utilizes the ability of a 3C Protease (derived from a HRV rhinovirus-14, EC: 3.4.22.28) to cleave a chromogenic peptide substrate to release a chromophore (pNA) which can be easily quantified using a 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-pNA $\xrightarrow{\text{HRV 3C Protease}}$ Cleaved substrate + pNA (Absorbance) (405 nm)

HRV 3C Substrate-pNA $\xrightarrow[+\text{ HRV 3C Protease Inhibitor}]{\text{HRV 3C Protease}}$ Decrease in Absorbance/No Absorbance

## II. Applications:

- Screening/studying/characterizing inhibitors of HRV 3C Protease.

## III. Kit Contents:

| Components | K215-100 | Cap Code | Part Number |
|---|---|---|---|
| HRV 3C Protease Assay Buffer | 25 ml | WM | K215-100-1 |
| HRV 3C Protease | 2 x 100 µl | Red | K215-100-2 |
| HRV 3C Protease Substrate | 500 µl | Brown | K215-100-3 |
| HRV 3C Protease Inhibitor (2 mM) | 20 µl | Purple | K215-100-4 |

## IV. User Supplied Reagents and Equipment:

- 96-well clear plate.
- Multi-well spectrometer.

## V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **HRV 3C Protease Assay Buffer:** Store at 4°C. Bring to room temperature before use.
- **HRV 3C Protease:** Aliquot and store at -80°C for long term. Avoid repeated freeze/thaw. Each vial contains enough enzyme solution for 50 assays.

## VI. HRV 3C Protease Inhibitor Screening Protocol:

1. **HRV 3C Protease Enzyme Solution Preparation**: For each well, prepare 50 µl of HRV 3C Protease enzyme solution.

   48 µl HRV 3C Protease Assay Buffer
   2 µl HRV 3C Protease enzyme

   Mix well and add 50 µl/well into desired wells in a 96-well microtiter plate.

2. **Screening Compounds, Inhibitor Control & Blank Control Preparations:** Dissolve test inhibitors into proper solvent. Dilute to 10X the desired test concentration with HRV 3C Protease Assay Buffer. Add 10 µl diluted test inhibitors (Sample, I) or HRV 3C Protease Assay Buffer (Enzyme Control, EC) into HRV 3C Protease enzyme containing wells. For Inhibitor Control (IC), add 1 µl HRV 3C Protease Inhibitor and 9 µl HRV 3C Protease Assay Buffer into HRV 3C Protease enzyme well(s). Incubate at room temperature for 15 min.

   **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 (SC).

3. **HRV 3C Protease Substrate Preparation:** For each well, prepare 40 µl of the substrate solution:

   35 µl HRV 3C Protease Assay Buffer
   5 µl HRV 3C Protease Substrate

   Mix & add 40 µl of HRV 3C Protease Substrate solution into Enzyme Control, Inhibitor Control, solvent control & sample wells. Mix well.

4. **Measurement:** Immediately, start measuring the absorbance at 405 nm (A405) in a kinetic mode for upto 1-2 h min at room temperature. Choose two time points (T1 & T2) where the corresponding absorbance is in a linear range. Calculate ΔA405 and ΔT.

5. **Calculations**: Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔA405 with the time ΔT ($T_2$-$T_1$).

$$\% \textbf{ Relative Inhibition} = \frac{\textbf{Slope of EC} - \textbf{Slope of I}}{\textbf{Slope of EC}} \times \textbf{100}$$

**Note**:

- If Solvent Control (SC) values are significantly different from the EC, use these values in the equation above.
- Irreversible inhibitors that inhibit the HRV 3C Protease activity completely at the tested concentration will have ΔA405 = 0 and thus the % Relative Inhibition will be 100%.

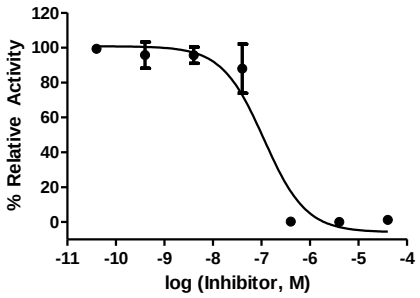


**Figure**: Inhibition of HRV 3C Protease activity by HRV 3C Protease Inhibitor (**$IC_{50}$ = 0.12 µM**). Assay was performed following the kit protocol.

## VII. RELATED PRODUCTS:

TEV Protease Activity Fluorometric Assay Kit (K842)
HIV-1 Protease Inhibitor Screening Kit (K826)
HIV-2 Protease Activity Assay Kit (Fluorometric) (K843)
Cathepsin L Activity Fluorometric Assay Kit (K142)
Cathepsin L (Cleaved) Antibody (3741)
Cathepsin L Blocking Peptide (3192BP)
Cathepsin B (1021)
Cathepsin B Antibody (3190)
Cathepsin D (1022)
Cathepsin D Antibody (3191R)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)
Cathepsin F Blocking Peptide (3371BP)
Cathepsin G Antibody (3370)
Cathepsin G Substrate (2206)
Cathepsin G Activity Fluorometric Assay Kit (K146)
Cathepsin H Activity Fluorometric Assay Kit (K145)
Procathepsin K, human recombinant (1026)
Procathepsin K, rat recombinant (1029)
Cathepsin K Blocking Peptide (3588BP, 3368BP)
Cathepsin S Activity Fluorometric Assay Kit (K144)
Cathepsin S Antibody (3366, 3366R)
EZCut™ TEV Protease, Recombinant (7847)
Active HIV1 Protease Recombinant (GST-tagged) (7849)
Active HIV-2 Protease Recombinant (GST-tagged) (7851)
HIV-1 Protease Activity Assay Kit (Fluorometric) (K825)
Cathepsin L (Cleaved) Blocking Peptide (3741BP)
Cathepsin L, human recombinant (1135)
Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin B Inhibitor Screening Kit (K147)
Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin D Blocking Peptide (3191RBP)
Cathepsin F Antibody (3371)
Cathepsin G Activity Assay Kit, Fluorometric (K146)
Cathepsin G Inhibitor (1982)
Cathepsin G, human neutrophil (4713)
Cathepsin H (1023)
Cathepsin K Activity Fluorometric Assay Kit (K141)
Procathepsin K, mouse recombinant (1027)
Cathepsin K Antibody (3588, 3368)
Human CellExp™ Cathepsin S, human recombinant (7277)
Cathepsin S Inhibitor Screening Kit (K149)
Cathepsin S Blocking Peptide (3366R)

***FOR RESEARCH USE ONLY! Not to be used on humans.***