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HRV 3C Protease Activity Assay Kit (Colorimetric)

rev 05/21

(Catalog # K214-100; 100 assays, Store kit at -80 XNW°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 Activity Assay 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 (*p*NA) which can be easily quantified using a microplate reader. This assay kit is simple, rapid and can detect HRV 3C Protease activity as low as 50 ng in samples and of purified proteins.

HRV 3C Substrate-*p*NA $\xrightarrow{\textbf{HRV 3C Protease}}$ Cleaved substrate + *p*NA (OD 405 nm)

II. Applications:

- Detection of HRV 3C Protease activity in samples
- Determine activity of purified HRV 3C Protease

III. Kit Contents:

Components	K214-100	Cap Code	Part Number
HRV 3C Protease Assay Buffer	25 ml	WM	K214-100-1
HRV 3C Protease (10 µg, Positive Control)	10 µl	Green	K214-100-2
HRV 3C Protease Substrate	500 µl	Brown	K214-100-3
*p*NA Standard (0.1 M)	20 µl	Yellow	K214-100-4

IV. User Supplied Reagents and Equipment:

- 96-well clear well plate.
- Multi-well spectrometer.
- **Optional:** BCA Protein Assay Kit - Reducing Agent Compatible **(Cat. # K818-1000, K819-250 or equivalent).**

V. Storage Conditions and Reagent Preparation:

Store the kit at -80 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the assay.

- **HRV 3C Protease Assay Buffer:** Store at 4 °C. Bring to room temperature (RT) before use.
- **HRV 3C Protease (Positive Control):** Divide into aliquots and store at -80 °C for long term. Avoid repeated freeze/thaw cycles.

VI. HRV 3C Protease Activity Assay Protocol:

1. **Standard Curve Preparation:** Dilute 5 µl of 0.1 M *p*NA Standard with 95 µl HRV 3C Protease Assay Buffer to obtain 5 mM of *p*NA Standard. Mix well. Add 0, 2, 4, 6, 8, and 10 µl of diluted Standard Solution into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with HRV 3C Protease Assay Buffer to generate 0, 10, 20, 30, 40, and 50 nmol/well of *p*NA Standard respectively. Mix well. Measure the absorbance at 405 nm in end point mode.
2. **Sample preparation Notes:**
   a. Measure the amount of protein in your sample using BCA Protein Assay Kit - Reducing Agent Compatible (**Cat. K818-1000, K819-250** or equivalent).
   b. Optional: For samples with potential background, prepare parallel sample well(s) as sample background control. Use same amount of the sample or purified enzyme as in the sample well. Adjust the final volume to 100 µl with HRV 3C Protease Assay Buffer.
3. **Positive Control:** Mix 18 µl of HRV 3C Protease Assay Buffer per 2 µl of the HRV 3C Protease positive control to obtain 20 µl of 100 ng/µl enzyme solution.
4. **Reaction Mix:** Prepare sample, Positive Control and reagent background wells as mentioned below:

	Sample	Reagent Background Control	Positive Control	Sample background Control
Sample	5-20 µl	-	-	5-20 µl
HRV 3C Protease (100 ng/µl)	-	-	5-20 µl	-
HRV 3C Protease Substrate	5 µl	5 µl	5 µl	-
HRV 3C Protease Assay Buffer	**Make up the volume to 100 µl in all mixtures**			

Mix well by pipetting up and down.

Note: Don't add Substrate mix to the Sample Background Control.

4. **Measurement:** Immediately, start measuring the absorbance at 405 nm ($A_{405\ nm}$) in a kinetic mode for up to 1-2 hr at RT. Choose two time points (T1 & T2) where the corresponding absorbance is in a linear range. Calculate $\Delta \mathbf{A}_{405\ nm}$ and ΔT.
5. **Calculations**: Subtract 0 Standard reading from all readings. Plot the *p*NA Standard Curve. Apply sample's $\Delta \mathbf{A}_{405\ nm}$ to *p*NA Standard Curve to obtain corresponding nmol of product formed (**B**, in nmol) and calculate the activity of HRV 3C Protease in the sample as:

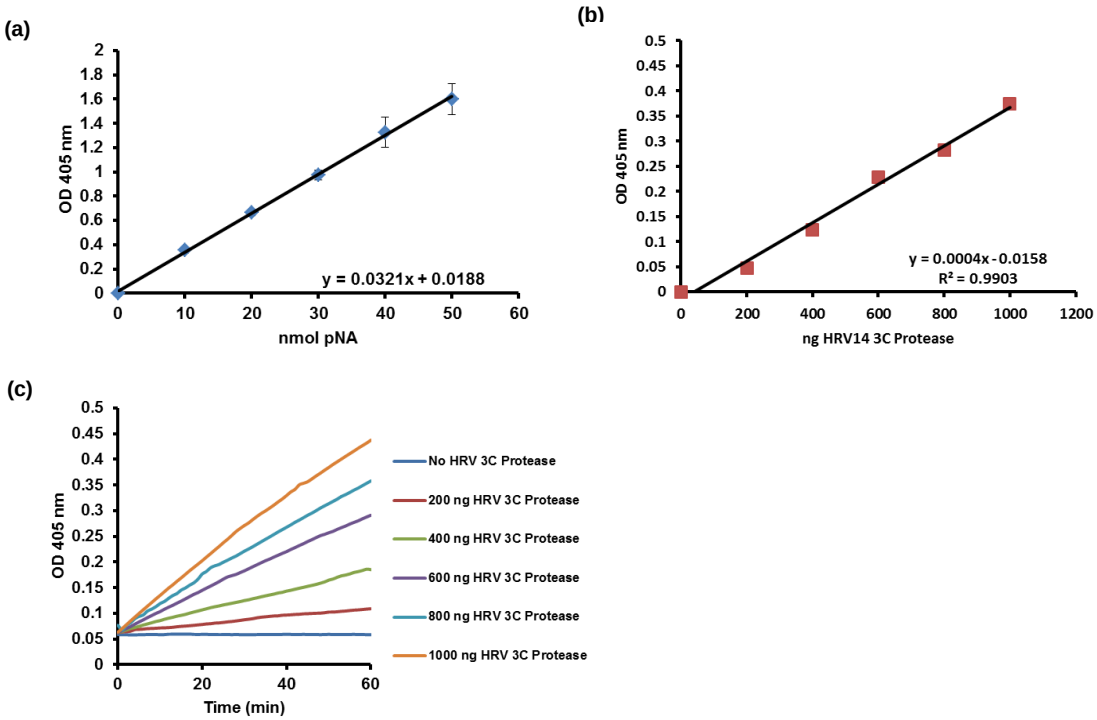
$$\textit{Sample}\ \textbf{HRV 3C Protease Activity} = \frac{B}{\Delta T \times V} \times \textbf{Dilution Factor} = \frac{\textbf{nmol}}{\textbf{min}}/\textbf{ml} \quad \textbf{= mU/ml}$$

Optional:

$$\textit{HRV 3C}\ \textbf{Protease Activity Per mg of Protein} = \frac{B}{\Delta T \times M} \times \textbf{Dilution Factor} = \frac{\textbf{nmol}}{\textbf{min}}/\textbf{mg} \quad \textbf{= mU/ml}$$

Where: **B** = Amount of product calculated from the *p*NA Standard Curve (nmol)
V = Sample volume initially added into the reaction well (ml)
M = Amount of protein in the sample (mg)
Δ**T** = reaction time (min)

Unit Definition: 1 Unit is defined as the amount of HRV 3C protease which can cleave 1 µmol of substrate/min under the assay conditions.



Figures: *p*NA Standard Plot (10-50 pmol, **a**) kinetics progress curves (**b**) calculated change in OD 405 nm (after 1 hr). **c**) for different amounts of HRV 3C Protease as a positive control are presented. Assays were performed following the kit protocol.

VII. Related Products:

HRV 3C Protease Inhibitor Screening Kit (K215)
Active HIV-2 Protease Recombinant (GST-tagged) (7851)
TEV Protease Inhibitor Screening Kit (K843)
HIV-1 Protease Inhibitor Screening Kit (K826)
Cathepsin L Activity Fluorometric Assay Kit (K142)
Cathepsin B Inhibitor Screening Kit (K147)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)
Cathepsin G Activity Fluorometric Assay Kit (K146)
Cathepsin K Activity Fluorometric Assay Kit (K141)
Cathepsin S Inhibitor Screening Kit (K149)
HIV-1 Protease Activity Assay Kit (Fluorometric) (K825)
HIV-2 Protease Activity Assay Kit (Fluorometric) (K842)
EZCut™ TEV Protease, Recombinant (7847)
Active HIV1 Protease Recombinant (GST-tagged) (7849)
Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin G Activity Assay Kit, Fluorometric (K146)
Cathepsin H Activity Fluorometric Assay Kit (K145)
Cathepsin S Activity Fluorometric Assay Kit (K144)

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