CREATIVE BIOMART®
Assay Kit

# HRV 3C Protease Activity Assay Kit (Colorimetric)

## Product Information

**Common Name**

Protease

**Cat.No.** Kit-1077

**Product Overview**

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 (pNA) 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.

**Description**

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.

**Storage**

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 before use.
HRV 3C Protease (Positive Control): Aliquot and store at -80°C for long term. Avoid repeated freeze/thaw cycles.

**Shipping**

Dry Ice

**Size**

100 assays

**Kit Components**

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# HRV 3C Protease Activity Assay Kit (Colorimetric)

HRV 3C Protease Assay Buffer, 25 ml
HRV 3C Protease (10 µg, Positive Control), 10 µl
HRV 3C Protease Substrate, 500 µl
pNA Standard (0.1 M), 20 µl

**Materials Required but Not Supplied**

96-well clear well plate.
Multi-well spectrometer.
Optional: BCA Protein Assay Kit - Reducing Agent Compatible

**Assay Protocol**

1. Standard Curve Preparation: Dilute 5 µl of 0.1 M pNA Standard with 95 µl HRV 3C Protease Assay Buffer to obtain 5 mM of pNA 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 pNA Standard respectively. Mix well. Measure the absorbance at 405 nm (A405) in an end point mode.
2. Sample preparation Notes:
a. Measure the amount of protein in your sample using BCA Protein Assay Kit - Reducing Agent Compatible.
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 | - |
| Substrate | 5 µl | 5 µl | 5 µl | - |
| Assay Buffer | Make up the volume to 100 µl in all mixtures | | | |

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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 (A405) in a kinetic mode for up to 1-2 hr at room temperature. Choose two time points (T1 & T2) where the corresponding absorbance is in a linear range. Calculate ΔA405 and ΔT.

5. Calculations: Subtract 0 Standard reading from all readings. Plot the pNA Standard Curve. Apply sample's ΔA405 to pNA Standard Curve to obtain corresponding nmol of product formed (B, in nmol) and calculate the activity of HRV 3C Protease in the sample as:

Sample HRV 3C Protease Activity= B/(ΔT x V) x Dilution Factor = (nmol/min)/ml= U/ml

Optional: HRV 3C Protease Activity per mg of protein= B/(ΔT x M) x Dilution Factor = (nmol/min)/mg= U/ml

Where: B = Amount of product calculated from the pNA Standard Curve (nmol)

V = Sample volume initially added into the reaction well (ml)

M = Amount of protein in the sample (mg)

ΔT = reaction time (min)

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