



HIV-2 Protease Activity Assay Kit (Fluorometric)

8/18

(Catalog #K845 -100; 100 assays, Store kit at -80°C)

I. Introduction:

Human Immunodeficiency Virus (HIV) is the cause of the Acquired Immunodeficiency Syndrome (AIDS). Infection with HIV-2 virus causes immunosuppression, as well as AIDS, characterized by the same signs, symptoms and opportunistic infections as HIV-1. However, HIV-2-associated AIDS may often be associated with lower viral load levels than HIV-1. Aspartyl HIV-2 Protease (retropepsin) is an essential enzyme for the life-cycle of HIV virus. Expressed as a part of the Gag-Pol polyprotein complex in the infected cells, it is auto-catalytically released upon formation of an immature viral particle. The enzyme subsequently cleaves the other parts of viral polyproteins resulting in the maturation of the virus. In HIV-infected patients the enzyme is subjected to intensive mutagenesis and mutants resistant to applied medicines are produced as a result of the selection pressure. BioVision's HIV-2 Protease Activity assay kit utilizes the ability of active HIV-2 Protease to cleave a synthetic peptide substrate to release the free fluorophore which can be easily quantified (Ex/Em = 330/450 nm) using a fluorometer or fluorescence microplate reader. This assay is simple, rapid and can detect HIV-2 protease activity in samples and of purified enzyme.



II. Applications:

- Detect activity of recombinant HIV-2 Protease
- Determine activity of purified HIV-2 Protease

III. Kit Contents:

| Components | K845-100 | Cap Code | Part Number |
|-----------------------------------|----------|----------|-------------|
| HIV-2 Protease Assay Buffer | 25 ml | WM | K845-100-1 |
| HIV-2 Protease Dilution Buffer | 1 ml | Clear | K845-100-2 |
| HIV-2 Protease Substrate | 0.2 ml | Red | K845-100-3 |
| HIV-2 Protease (Positive Control) | 20 µl | Orange | K845-100-4 |
| Fluorescence Standard (10 mM) | 20 µl | Yellow | K845-100-5 |

IV. User Supplied Reagents and Equipment:

- 96-well white plate.
- Multi-well spectrophotometer.
- BCA Protein Assay Kit - Reducing Agent Compatible (**Cat. # K818-1000/ K819-250** or equivalent).

V. Storage Conditions and Reagent Preparation:

Store 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.

- **HIV-2 Protease Assay and Dilution Buffers:** Bring to room temperature before use. Store at -20°C.
- **HIV-2 Protease:** Add 20 µl of HIV Protease Dilution Buffer to the vial. Aliquot and store at -80°C. Avoid repeated freeze/thaw.

VI. HIV-2 Protease Activity Assay Protocol:

1. **Standard Curve Preparation:** To obtain 1 mM of Fl. Standard, dilute 10 µl of 10 mM Fl. Standard with 90 µl HIV-2 Protease Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of diluted Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with HIV-2 Protease Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of Fl. Standard respectively. Mix well. Measure fluorescence (Ex/Em = 330/450 nm).

Notes:

- a. Measure the amount of protein of the 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 HIV-2 Protease Assay Buffer.
2. **Reaction Mix:** Prepare sample, Positive Control and reagent background wells as mentioned below:

| | Sample | Reagent Background Control | Positive Control |
|--|--|----------------------------|------------------|
| Sample | 2-50 µl | - | - |
| HIV-2 Protease (Positive Control) | - | - | 2-8 µl |
| HIV-2 Protease Assay Buffer | Make up the volume to 98 µl in all 3 mixtures | | |
| HIV-2 Protease Substrate | 2 µl | 2 µl | 2 µl |

Mix well by pipetting up and down.

Note: Don't add substrate mix to the sample Background Control and Standard wells.

3. **Measurement:** Measure fluorescence (Ex/Em = 330/450 nm) of the samples and the controls in a kinetic mode for 1-3 hr at 37°C. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU₁ and RFU₂) for the sample and substrate background. Subtract background Δ RFU from sample Δ RFU.

4. **Calculations:** Measure the fluorescence of the standards in an end point mode. Subtract 0 Standard reading from all readings. Plot the FI. Standard Curve. Apply sample's Δ RFU to FI. Standard Curve to obtain corresponding nmol of product formed (**B**, in nmol) and calculate the activity of HIV-2 Protease in the sample as:

$$\text{Sample HIV - 2 Protease Activity} = \frac{B}{\Delta T \times M} \times \text{Dilution Factor} = \frac{\text{nmol}}{\text{min}} / \text{mg} = \text{mU/mg}$$

Where: **B** = nmol of product formed from the FI. Standard Curve (nmol)
M = Amount of protein in the sample (mg)
 ΔT = reaction time (min)

Unit Definition: 1 unit is defined as the amount of HIV-2 protease which can cleave 1 μ mol of substrate/min.

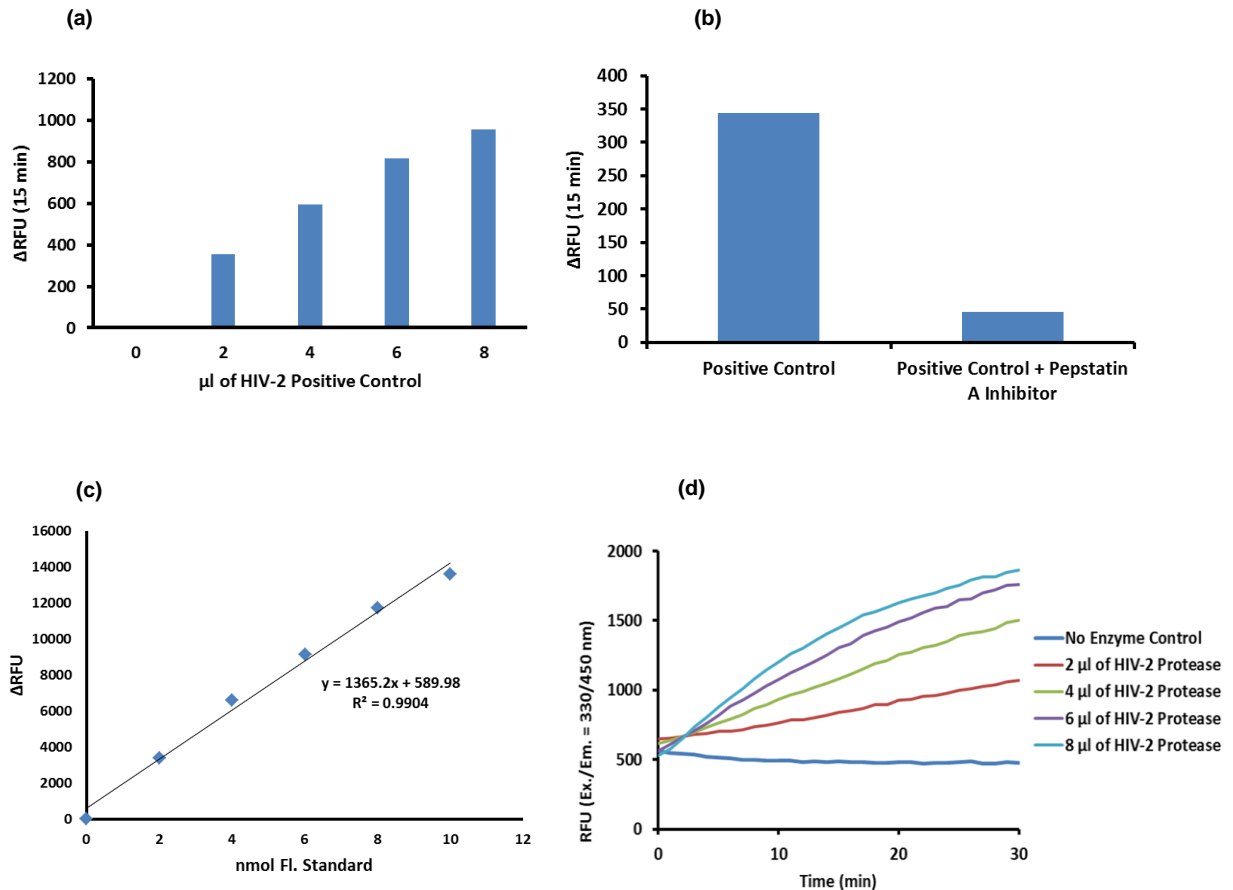


Figure: Measurement of HIV-2 Protease activity using HIV-2 Protease as a positive control (a) and its inhibition by Pepstatin A inhibitor (Cat # 1732) (b). FI. Standard plot (c) Progressive activity curves using different amounts of HIV-2 Protease (Positive Control) with time (d). Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

Active HIV-2 Protease Recombinant (GST-Tagged) (7851)
TEV Protease Activity Assay Kit (K842)
EZCut™ TEV Protease, Recombinant (7847)
Cathepsin B Inhibitor Screening Kit (Fluorometric) (K147)
Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)
Cathepsin G Inhibitor Screening Kit (Colorimetric) (K162)
Cathepsin H Activity Fluorometric Assay Kit (K145)
Cathepsin S Activity Fluorometric Assay Kit (K144)

HIV-1 Protease Inhibitor Screening Kit (Fluorometric) (K826)
HIV-1 Protease Activity Assay Kit (Fluorometric) (K825)
TurboTEV Protease, Recombinant (9205)
Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin L (Cleaved) Blocking Peptide (3741BP)
Cathepsin G Activity Colorimetric Assay Kit (K146)
BCA Protein Assay Kit - Reducing Agent Compatible (K818, K819)
Cathepsin K Activity Fluorometric Assay Kit (K141)
Cathepsin S Inhibitor Screening Kit (K149)

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