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HIV-1 Protease Activity Assay Kit (Fluorometric)

rev 02/21

(Catalog # K825-100; 100 assays, Store kit at -80 °C)

I. Introduction:

Human Immunodeficiency Virus (HIV) is the cause of the Acquired Immunodeficiency Syndrome (AIDS). HIV-1 protease is a retroviral aspartyl protease (retropepsin) that is essential for the life-cycle of the virus as it cleaves newly synthesized polyproteins at the appropriate places to create the mature protein components of an infectious HIV virion. Without effective HIV1 protease, HIV virions remain non-infectious. **BioVision's HIV-1 Protease Activity Assay Kit** utilizes the ability of active HIV-1 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 kit is simple, rapid and can detect HIV-1 protease activity in samples and of purified enzyme.

Fl-Substrate $\xrightarrow{\text{HIV-1 Protease}}$ Cleaved substrate + Fl (Fluorescence) (Ex/Em = 330/450 nm)

II. Applications:

- Detect the activity of recombinant or purified HIV-1 protease.

III. Kit Contents:

Components	K825-100	Cap Code	Part Number
HIV-1 Protease Assay Buffer	25 ml	WM	K825-100-1
HIV-1 Protease Dilution Buffer	1 ml	Clear	K825-100-2
HIV-1 Protease Substrate	0.2 ml	Red	K825-100-3
HIV-1 Protease (Positive Control)	7 µl	Blue	K825-100-4
Fluorescence Standard (10 mM)	20 µl	Yellow	K825-100-5

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom.
- 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-1 Protease Assay and Dilution Buffers:** Bring to room temperature before use. Store at -20 °C.
- **HIV-1 Protease:** Add 13 µl of HIV Protease Dilution Buffer to the vial. Aliquot and store at -80 °C. Avoid repeated freeze/thaw.

VI. HIV1 Protease Activity Assay Protocol:

1. **Standard Curve Preparation:** To obtain 0.1 mM of Fluorescence Standard, dilute 10 µl of 10 mM Fluorescence Standard with 990 µl HIV-1 Protease Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of diluted Fluorescence Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with HIV-1 Protease Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, and 1 nmol/well of Fluorescence Standard respectively. Mix well. Measure the 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 a parallel sample well(s) as sample background control. Use the same amount of the sample or purified enzyme as in the sample well. Adjust the final volume to 100 µl with HIV-1 Protease Assay Buffer.

2. **Reaction Mix:** Prepare sample, Positive Control and reagent background wells as mentioned below:

	Sample	Reagent Background Control	Positive Control
Sample	2-20 µl	-	-
HIV-1 Protease (Positive Control)	-	-	2-10 µl
HIV-1 Protease Assay Buffer	**Make up the volume to 98 µl in all 3 mixtures**		
HIV-1 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 (RFU_1 and RFU_2) for the sample and substrate background. Subtract background ΔRFU from sample ΔRFU.

4. **Calculations**: Measure the fluorescence of the Standards in an end point mode. Subtract 0 Standard reading from all readings. Plot the Fluorescence Standard Curve. Apply sample's ΔRFU to Fl. Standard Curve to obtain corresponding nmol of product formed (**B**, in nmol) and calculate the activity of HIV-1 Protease in the sample as:

$$\textit{Sample}\ \textbf{HIV}-\textbf{1 Protease Activity} = \frac{\boldsymbol{B}}{\boldsymbol{\Delta T}\times\boldsymbol{M}} \times \textbf{Dilution Factor} = \frac{\textbf{nmol}}{\textbf{min}}/\textbf{mg} \quad \textbf{= mU/mg}$$

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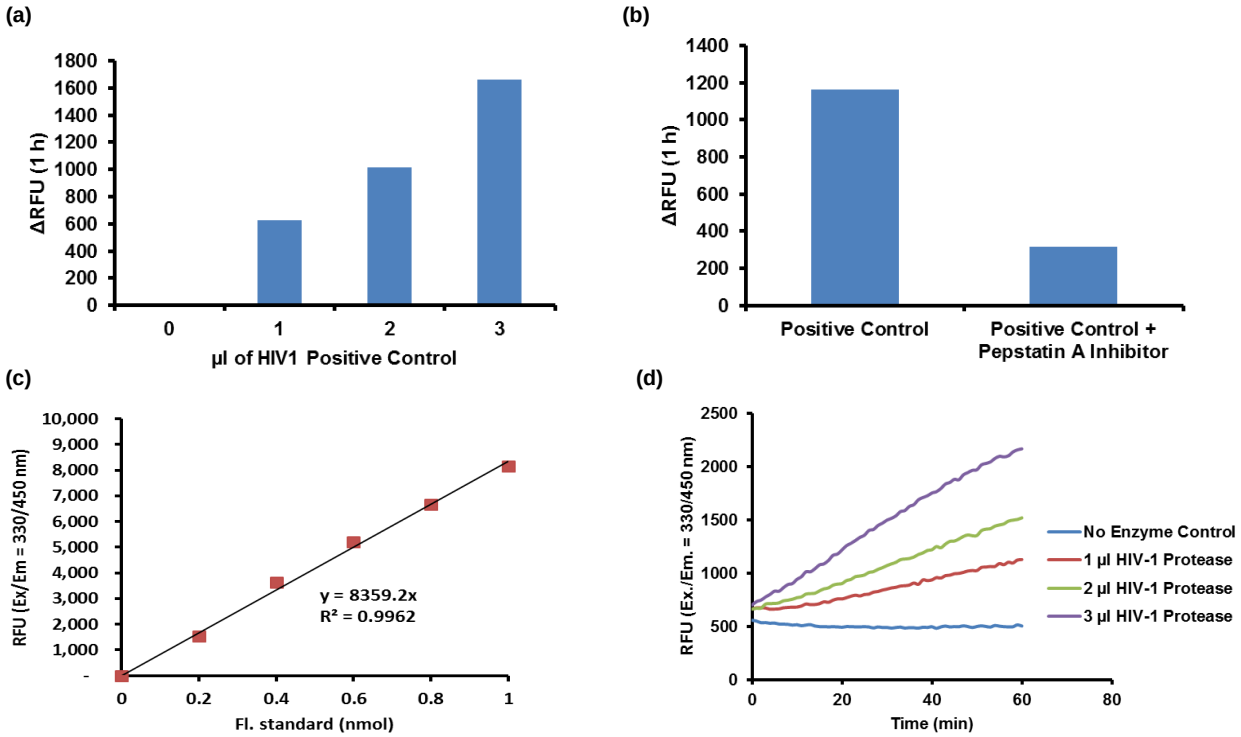
Where: **B** = nmol of product formed from the Fluorescence Standard Curve (nmol)
M = Amount of protein in the sample (mg)
Δ**T** = reaction time (min)

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



Figures: Measurement of HIV1 Protease activity using **(a).** HIV-1 Protease as Positive Control. **(b).** Inhibition by Pepstatin A inhibitor (Cat # 1732). **(c).** Fluorescence Standard Curve. **(d).** Progressive activity curves using different amounts of HIV-1 Protease (Positive Control) with time. Assays were performed following the kit protocol.

VII. Related Products:

Active HIV1 Protease Recombinant (GST-Tagged) (7849)
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)
TEV Protease Inhibitor Screening Kit (K843)
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)

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