ab284560 – HIV-2 Protease Activity Assay Kit (Fluorometric)

For the screening of potential HIV-2 Protease Activity.
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284560

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer XLVIII/HIV-2 Protease Assay Buffer	25 ml	-80°C
Dilution Buffer VII/HIV-2 Protease Dilution Buffer	1 ml	-80°C
HIV Protease Substrate/HIV-2 Protease Substrate	0.2 ml	-80°C
HIV-2 Protease Positive Control/HIV-2 Protease (Positive Control)	20 µl	-80°C
Fluorescence Standard/Fluorescence Standard (10 mM)	20 µl	-80°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well white plate
- Multi-well spectrophotometer.
- BCA Protein Assay Kit - Reducing Agent Compatible (ab207003, ab207004 or equivalent)

Reagent Preparation

- 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 Positive Control/HIV-2 Protease: Add 20 µl of HIV Protease Dilution Buffer to the vial. Aliquot and store at -80°C. Avoid repeated freeze/thaw.

Assay Protocol

1. Standard Curve Preparation: To obtain 1 mM of Fl. Standard, dilute 10 µl of 10 mM Fl. Standard with 90 µl Assay Buffer XLVIII/HIV-2 Protease Assay Buffer.
2. 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 Assay Buffer XLVIII/HIV-2 Protease Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of Fl. Standard respectively. Mix well.
3. Measure fluorescence (Ex/Em = 330/450 nm).

Δ Notes:
a. Measure the amount of protein of the sample using BCA Protein Assay Kit - Reducing Agent Compatible (ab207003, ab207004 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 Assay Buffer XLVIII/HIV-2 Protease Assay Buffer.

Reaction Mix:

1. 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/HIV-2 Protease (Positive Control)	-	-	2-8 µl
Assay Buffer XLVIII/HIV-2 Protease Assay Buffer	**Make up the volume to 98 µl in all 3 mixtures**		
HIV Protease Substrate/HIV-2 Protease Substrate	2 µl	2 µl	2 µl

2. Mix well by pipetting up and down.

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

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 (T1 & T2) 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 ΔRFU from sample ΔRFU.

Calculation:

Measure the fluorescence of the standards in an end point mode. Subtract 0 Standard reading from all readings.
Plot the Fl. 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-2 Protease in the sample as:

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

Where: **B** = nmol of product formed from the Fl. Standard Curve (nmol)
M = Amount of protein in the sample (mg)

ΔT = reaction time (min)

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)