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ab211105
HIV-1 Protease Activity Assay Kit

For the rapid, sensitive and accurate measurement of HIV-1 Protease activity in a variety of samples.

This product is for research use only and is not intended for diagnostic use.

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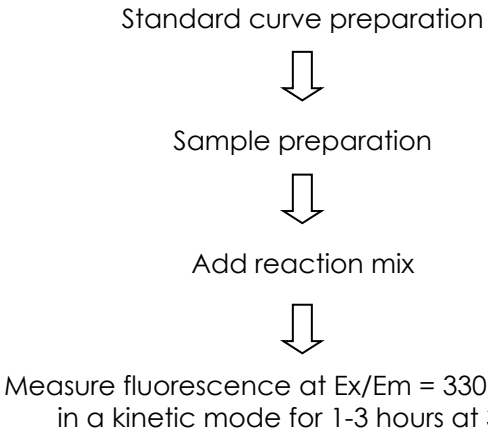
1. Overview

HIV-1 Protease Activity Assay Kit (Fluorometric) (ab211105) provides a convenient and simple method for detecting HIV-1 Protease activity in mammalian tissue samples and from purified proteins. The assay kit is based on the ability of active HIV-1 protease to cleave a synthetic peptide substrate to release the free fluorophore which can be easily quantified at Ex/Em = 330/450 nm using a fluorescence microplate reader.

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.

2. Protocol Summary



**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -80°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Fl. Standard (10 mM in DMSO)	20 µL	-80°C	-80°C
HIV-1 Protease (Positive Control)	7 µL	-80°C	-80°C
HIV-1 Protease Assay Buffer	25 mL	-80°C	-20°C
HIV-1 Protease Dilution Buffer	1 mL	-80°C	-20°C
HIV-1 Protease Substrate	200 µL	-80°C	-80°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 330/450 nm
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably white
- Dounce homogenizer (if using tissue)
- BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Fl. Standard (10 mM in DMSO) (20 µL):

Ready to use as supplied. Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored below -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -80°C.

9.2 HIV-1 Protease (Positive Control) (7 µL):

Add 13 µL of HIV Protease Dilution Buffer to the vial. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw.

9.3 HIV-1 Protease Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.4 HIV-1 Protease Dilution Buffer (1 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.5 HIV-1 Protease Substrate (200 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -80°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 0.1 mM Fl. working standard solution by adding 10 µL of 10 mM Fl. Standard to 990 µL of HIV-1 Protease Assay Buffer.

10.2 Using 0.1 mM Fl. working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Fl. 1 mM standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount Fl. in well (nmol/well)
1	0	300	100	0
2	2	298	100	0.2
3	4	296	100	0.4
4	6	294	100	0.6
5	8	292	100	0.8
6	10	290	100	1

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

10.3 Immediately measure fluorescence in an end point mode program on a microplate reader at Ex/Em = 330/450 nm.

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 HIV-1 Infected Tissue lysates:

11.1.1 Harvest the amount of tissue necessary for each assay (initial recommendation ~ 5 – 10 mg).
11.1.2 Wash tissue in cold PBS.
11.1.3 Add100 µL HIV-1 Protease Assay Buffer to the tissue.
11.1.4 Keep on ice for 5 minutes.
11.1.5 Homogenize tissue in with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
11.1.6 Centrifuge sample for 10 minutes at 4°C at 16,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new tube.
11.1.8 Keep on ice.
11.1.9 Measure the amount of protein in the lysate using BCA Protein Assay kit (reducing agent compatible).

11.2 Purified protein:

Use directly. No sample preparation is required.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Plate Loading:

- Sample Background Control wells = 2-20 µL samples (adjust volume to 100 µL/well with Protease Assay Buffer).
- Sample wells = 2 – 20 µL samples (adjust volume to 98 µL/well with Protease Assay Buffer).
- Positive control = 2 – 10 µL HIV-1 Protease (adjust volume to 98 µL/well with Protease Assay Buffer).
- Reagent Background control wells = 98 µL Protease Assay

12.2 HIV-1 Protease Reaction:

12.2.1 Add 2 µL of HIV-1 Protease Substrate to Sample, positive control and reagent background control wells.

Δ Note: Do not add Substrate to the Sample Background Control wells.

12.2.2 Mix well by pipetting up and down.

The table bellows shows the reaction set up:

Component	Sample Bckg Control (µL)	Sample (µL)	Reagent Bckg Control (µL)	Positive Control (µL)
Sample	2-20	2-20	0	0
HIV-1 Protease (Positive Control)	0	0	0	2-10
HIV-1 Protease Assay Buffer	Up to 100 µL	Up to 98 µL		
HIV-1 Protease Substrate	0	2	2	2

12.3 Measurement:

12.3.1 Measure immediately fluorescence at Ex/Em = 330/450 nm on a microplate reader in a kinetic mode for up 1-3 hours at 37°C.

Δ Note: Incubation time depends on the HIV-1 Protease activity in the samples. We recommend measuring fluorescence in a kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the HIV-1 Protease activity of the samples.

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

13.1.1 Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings, read in end point mode. This is the corrected fluorescence.

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of HIV-1 Protease in the sample:

13.2.1 For all reaction wells, choose two points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding absorbance values at those points (RFU1 and RFU2).

13.2.2 Calculate ∆RFU as follows:

$$\Delta RFU = RFU2 - RFU1$$

13.2.3 If significant, subtract the sample background control from the sample reading.

13.2.4 Apply variation of fluorescence in the sample (∆RFU) to FI. Standard Curve to get B nmol of corresponding product formed during the reaction.

13.2.5 HIV-1 protease activity (nmol/min/mg or mU/mg) in the test sample is calculated as:

$$HIV1\ Protease\ Activity = \left(\frac{B}{\Delta T * M}\right) * D$$

Where:

B = amount of product calculated from the Fl. Standard Curve (nmol)

ΔT = linear phase reaction time T2 – T1 (min)

M = amount of protein in sample well (mg)

D = sample dilution factor

Unit definition:

1 Unit HIV-1 Protease activity = amount of HIV-1 protease which can cleave 1 µmol of substrate/min under the assay conditions.

14. Typical Data

Typical standard curve – data provided for **demonstration purposes** only. A new standard curve must be generated for each assay performed.

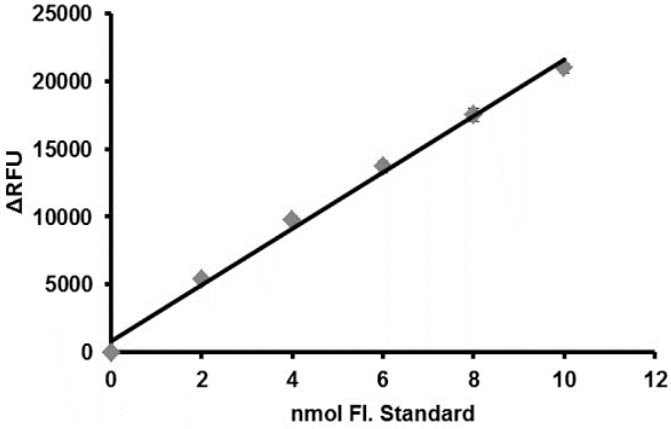


Figure 1. Typical Fl. standard calibration curve.

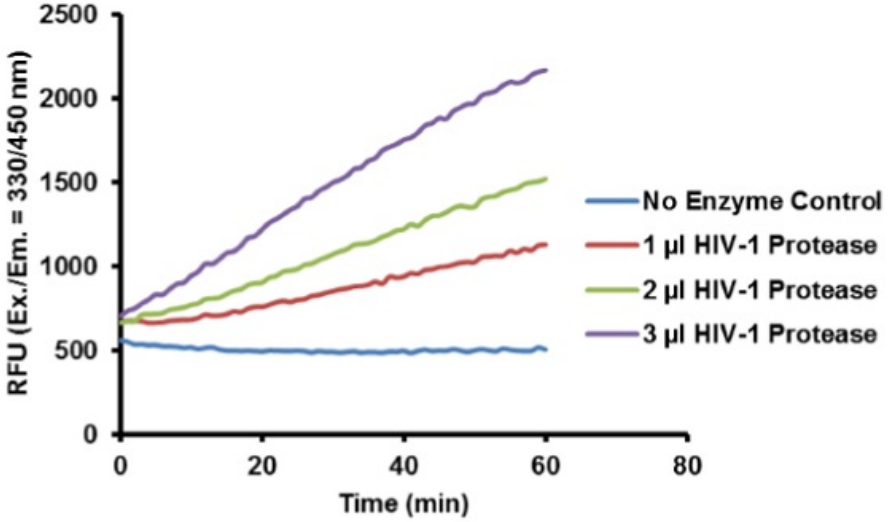


Figure 2. Kinetic progress curves observed for increasing amounts of HIV-1 Protease (positive control, included in the kit) through time.

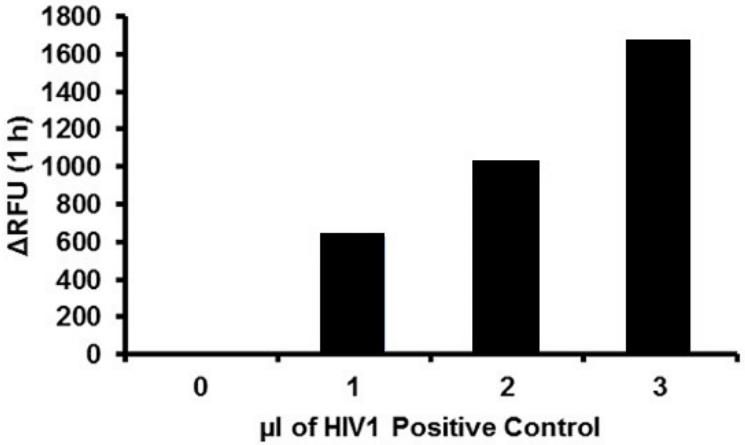


Figure 3. Measurement of HIV-1 Protease activity using increasing amount of HIV-1 Protease positive control included in the kit, calculated from data from figure 2.

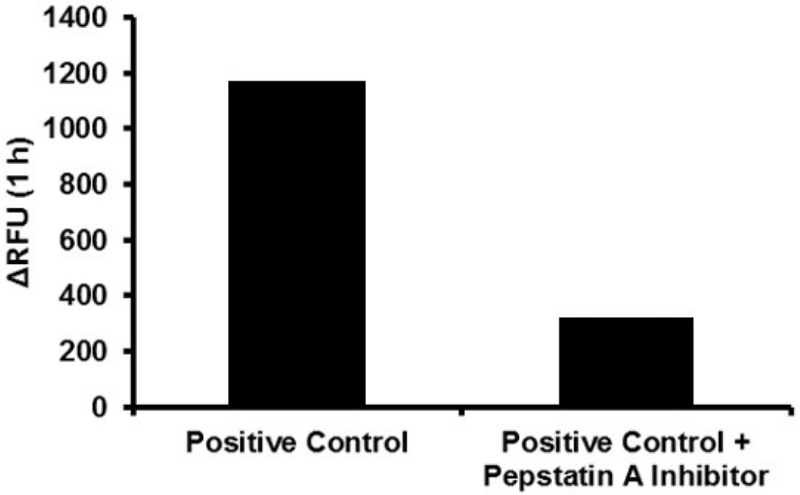


Figure 4. Measurement of HIV-1 Protease activity using HIV-1 Protease included in kit as positive control and activity of HIV-1 Protease in presence of Pepstatin A (protease inhibitor).

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare Fl. standard, positive control and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare Fl. standard dilution [2 – 10 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up sample plate in duplicate for standard (100 µL) and measure fluorescence of standard in a microplate reader at Ex/Em = 330/450 nm in end point mode.
- Set up background control samples (100 µL), reagent background control (98 µL), sample (98 µL) and positive control wells (98 µL).
- Add 2 µL of HIV-1 Protease Substrate to Sample, reagent background control and positive control wells.

Component	Sample Bckg Control (µL)	Sample (µL)	Reagent Bckg Control (µL)	Positive Control (µL)
Sample	2-20	2-20	0	0
HIV-1 Protease (Positive Control)	0	0	0	2-10
HIV-1 Protease Assay Buffer	Up to 100 µL	Up to 98 µL		
HIV-1 Protease Substrate	0	2	2	2

- Measure fluorescence immediately at Ex/Em = 330/450 nm in a kinetic mode for 1 – 3 hours at 37°C.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

17. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829