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ab211106 HIV-1 Protease Inhibitor Screening Kit (Fluorometric)

For the rapid, sensitive and accurate screening of potential HIV-1 Protease inhibitors.

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

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1. Overview

HIV-1 Protease Inhibitor Screening Kit (Fluorometric) (ab211106) provides a rapid, simple test suitable for high-throughput screening of HIV-1 Protease inhibitors. The assay is based on the ability of active HIV-1 protease to cleave a synthetic peptide substrate to release a free fluorophore, which can be easily quantified using a fluorescence microplate reader. In the presence of a HIV-1 Protease inhibitor, the cleavage of the substrate is reduced/abolished, resulting in decrease or total loss of the fluorescence.

This simple and high-throughput adaptable assay kit can be used to screen/study/characterize potential inhibitors of HIV-1 Protease.

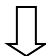
Fl Substrate —HIV-1 protease→ Cleaved substrate
(Fluorescence Ex/Em = 330/450 nm)

Fl Substrate —HIV-1 protease + Inhibitor→ Decrease in fluorescence/ No fluorescence

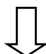
Human Immunodeficiency Virus (HIV) is the cause of the Acquired Immunodeficiency Syndrome (AIDS). HIV-1 protease is a retroviral aspartyl protease (retropepsin) that is essential for the life-cycle of the virus as it cleaves newly synthesized polyproteins at the appropriate places to create the mature protein components of an infectious HIV virion. Without effective HIV-1 protease, HIV virions remain non-infectious.

2. Protocol Summary

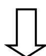
Screening compound & controls preparation



Enzyme and substrate solution preparation

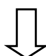


Add enzyme solution to wells.



Add substrate solution to wells

Incubate for 15 minutes at RT



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

**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)
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
HIV-1 Protease	2 x 1 vials	-80°C	-80°C
HIV-1 Protease Inhibitor (1 mM Pepstatin A in DMSO)	20 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
- 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 black

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

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

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

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

9.3 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.

9.4 HIV-1 Protease:

To each vial of HIV-1 Protease, add 70 µL of HIV-1 Protease Dilution Buffer as needed. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw. Use within two months.

9.5 HIV-1 Protease Inhibitor (1 mM Pepstatin A 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 inhibitor so that you have enough volume to perform the desired number of assays. Store at -80°C.

10. Sample Preparation

General sample information:

– Always prepare a fresh set of samples and controls for every use.

10.1 Screening Compounds:

10.1.1 Dissolve test compounds into proper solvent.

10.1.2 Dilute to 10X the desired test concentration with HIV-1 Protease Assay Buffer before use.

Δ Note: We suggest using different concentrations of test compounds if effective concentration is unknown.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.

Δ Note: Preferred final solvent concentration should not be more than 5% by volume. If solvent exceeds 5%, include solvent control to test the effect of the solvent on enzyme activity.

11.1 Set up Reaction wells:

- Screening sample compound wells (S) = 10 µL test compounds.
- Inhibitor Control wells (IC) = 1 µL Pepstatin A + 9 µL HIV-1 Protease Assay Buffer.
- Enzyme Control wells (EC) = 10 µL HIV-1 Protease Assay Buffer.
- OPTIONAL: Solvent control (SC) = 10 µL solvent.

11.2 Incubate plate at room temperature for 15 minutes.

11.3 Prepare HIV-1 Protease Enzyme Solution:

11.3.1 Prepare 80 µL of enzyme solution for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	HIV-1 Protease Solution (µL)
HIV-1 Protease Assay Buffer	78
Reconstituted HIV-1 Protease solution	2

11.3.2 Mix well and add 80 µL/well into each well.

11.4 HIV-1 Protease Substrate Solution:

11.4.1 Prepare 10 µL of the Substrate Solution mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	HIV-1 Protease Substrate Mix (µL)
HIV-1 Protease Assay Buffer	8
HIV-1 Protease Substrate	2

11.4.2 Mix and add 10 µL of HIV-1 Protease Substrate Solution into each well. Mix well.

11.5 Measurement:

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

12. Calculations

– Use only the linear rate for calculation.

12.1 Plot readings for each sample test compound (S), inhibitor control (IC) and enzyme control (EC).

12.2 Draw the line of the best fit to construct the curve (most plate reader software or Excel can do this step). Calculate the trend line equation (use the equation that provides the most accurate fit).

12.3 Choose two points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the absorbance (RFU1 and RFU2).

12.4 Calculate Slope (ΔRFU/ΔT) for all samples (S), Enzyme Control (EC) and Inhibitor control (IC), if desired, as follows:

$$\Delta RFU/\Delta T = (RFU2 - RFU1)/(T2 - T1)$$

12.5 Average the slope for each duplicate reading.

12.6 Calculate the % Relative Inhibitions as follows:

$$\%\,Relative\ Inhibition = \frac{Slope\ of\ EC - Slope\ of\ S}{Slope\ of\ EC}\ x\ 100$$

Δ **Note:** Irreversible inhibitors that inhibit HIV-1 Protease activity completely at the tested concentration will have ΔRFU = 0 and thus % Relative Inhibition will be 100%.

Δ **Note:** If RFU of Solvent Control(s) < RFU of Enzyme Control(s) = make a higher stock of test inhibitor or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent if possible.

If RFU of S < RFU of EC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. Typical Data

Data provided **for demonstration purposes only**.

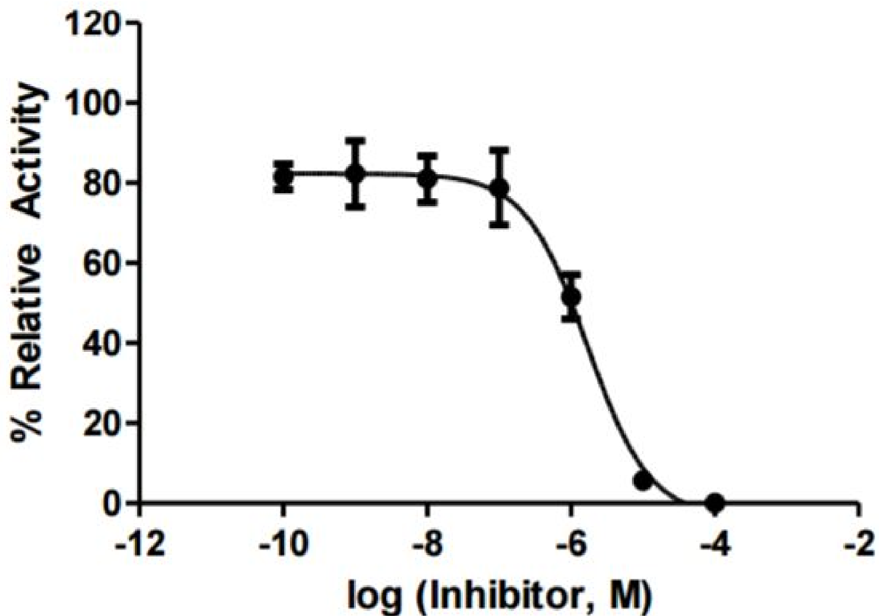


Figure 1. Typical inhibition curve of HIV-1 Protease activity by the protease inhibitor Pepstatin A (IC_{50} = 1.6 µM) Assay was performed following the kit protocol.

14. 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.

- Reconstitute enzyme (aliquot if necessary) and thaw components; get equipment ready.
- Prepare test compounds in suitable solvents; dilute if appropriate.
- Prepare HIV-1 Protease Solution (80 µL/well) by adding 2 µL of human Reconstituted HIV-1 Protease Solution to 78 µL of HIV-1 Protease Assay Buffer. Prepare a mix for all wells.
- Prepare HIV-1 Protease Substrate Solution (10 µL/well) by adding 2 µL of HIV-1 Protease Substrate to 8 µL of HIV-1 Protease Assay Buffer. Prepare a mix for all wells.
- Set up plate as follows:

Component	Sample (S) (µL)	Solvent control (SC) (µL)	Enzyme Control (EC) (µL)	Inhibitor Control (IC) (µL)
Test Compound	10	0	0	0
HIV-1 Protease Inhibitor (Pepstatin A)	0	0	0	1
Solvent test compound	0	10	0	0
Assay Buffer	0	0	10	9
Incubate plate at RT 15 minutes				
Add 80 µL HIV-1 Protease Solution				
Add 10 µL HIV-1 Protease Substrate				

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

15. 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
Assay with erratic readings	Pipetting errors	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
No fluorescence above background in inhibitor wells	Inhibitor concentration is too high	Reduce concentration of inhibitor and re-do assay
No inhibition seen in test compound wells	Inhibitor concentration is not high enough	Increase concentration of inhibitor and re-do assay
	Compound is not an inhibitor	Use another compound for your test

16. Notes

Technical Support

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