Enzymatic Suitability Assay of HIV PROTEASE

PRINCIPLE:

HIV Protease Substrate $\xrightarrow{\text{HIV Protease}}$ Product A + Product B

Abbreviations used:
HIV Protease Substrate = His-Lys-Ala-Arg-Val-Leu-p-nitro-Phe-
Glu-Ala-Nle-Ser-NH_2
Product A = His-Lys-Ala-Arg-Val-Leu
Product B = p-nitro-Phe-Glu-Ala-Nle-Ser-NH_2

CONDITIONS: T = 37°C, pH = 5.5, A_{215nm}

METHOD: HPLC Analysis of Proteolytic Cleavage Products

REAGENTS:

A. 50 mM Sodium Acetate Buffer, pH 5.5 at 37°C
(Prepare 10 mL in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, Adjust the pH to 5.5 at 37°C with 1 M HCl.)

B. 50 mM Sodium Acetate Buffer, pH 5.5 at 37°C with 1.085 M Glycerol, 1 mM Dithiothreitol, 1.0 M Urea, and 100 mM Ethylenediaminetetraacetic Acid Disodium (Refolding Buffer)
(Prepare 100 mL in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, Glycerol, Sigma Prod. No. G-9012, DL-Dithiothreitol, Sigma Prod. No. D-0632, Urea, Sigma Prod. No. U-1250, and Ethylenediaminetetraacetic Acid, Disodium Dihydrate, Sigma Stock No. ED2SS. Adjust the pH to 5.5 at 37°C with 1 M HCl.)

C. 1.5 mM HIV Protease Substrate in 50 mM Sodium Acetate, pH 5.5 at 37°C (Substrate Solution)
(Prepare 2 ml in Reagent A using HIV Protease Substrate,
Sigma Prod. No. H-5397.)

D. HIV Protease Solution
(Immediately before use, prepare a solution containing 0.05 mg/ml HIV Protease in Refolding Buffer (Reagent B).)

REAGENTS: (continued)

E. 0.1% Trifluoroacetic Acid in Acetonitrile/Water (3:1) (Solvent A)
(Prepare 500 ml in deionized water using Trifluoroacetic Acid, Sigma Prod. No. T-1647, Acetonitrile, Sigma Stock No. 27071-7, and deionized water.)

F. 0.1% Trifluoroacetic Acid in water (Solvent B)
(Prepare 500 ml in deionized water using Trifluoroacetic Acid, Sigma Prod. No. T-1647.)

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into a suitable tube:

Reagent D (Enzyme Solution)	0.09
Reagent C (Substrate Solution)	0.02

Mix gently and incubate for 120 minutes at 37°C. Remove tube and put in an ice bath to stop the reaction. Refrigerate until the time of HPLC analysis.

Step 2:

HPLC Conditions:

Column:	Vydac Reverse Phase C18, Particle size: 5 µm, 25 cm x 4.6 mm
Sample:	10 µL
Solvents:	A. 0.1% Trifluoroacetic Acid in Acetonitrile/Water (3:1) B. 0.1% Trifluoroacetic Acid in water
Gradient:	10% to 85% over 20 minutes
Flow Rate:	1.5 ml/min
Detection:	215 nm

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SUITABILITY:

Suitable for cleaving HIV substrate III. Yields 2 peaks which elute before the substrate peak when analyzed by reversed phase HPLC.

FINAL ASSAY CONCENTRATION:

In a 0.11 ml reaction mix, the final concentrations are 0.27 mM HIV Protease substrate III, 50 mM sodium acetate, 888 mM glycerol, 0.82 mM dithiothreitol, 818 mM urea, 82 mM ethylenediaminetetraacetic acid, 4.5 µg HIV protease.

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.