Revised: 19–August–2001

HIV Protease Substrate 1 (H-2930)

For Fluorometric Measurement of HIV Protease Activity

Quick Facts

Storage upon receipt:
- 4°C
- Desiccate
- Protect from light

Abs/Em: 340/490 nm

Introduction

Mammalian retroviruses encode a 10–12 kD aspartic protease (PR) that is expressed as part of the $PR^{gag\text{-}pol}$ precursor. Retroviral PR is required for processing of both the Pr^{gag} and $Pr^{gag\text{-}pol}$ precursor polyproteins at specific cleavage sites. These modifications are known to be required for maturation of infectious virus particles of human immunodeficiency virus 1 (HIV-1). Activity of retroviral PR has been measured by immunoblot analysis of the gag protein and its cleavage products, combined with HPLC or TLC analysis of synthetic peptide cleavage products. These methods are very labor intensive and do not permit kinetic measurement of enzyme activity.

A fluorometric method for measuring HIV-protease (HIV-PR) activity utilizing a synthetic peptide substrate for HIV-PR has been described by Wang and coworkers.[1,2] The sequence of this substrate includes the HIV-PR cleavage site, along with two covalently modified amino acid residues, one that has been linked to a fluorophore (5-(aminoethyl)aminonaphthalene sulfonate, EDANS) and the other to an acceptor chromophore (4′-dimethylaminoazobenzene-4-carboxylate, dabcyl). The acceptor chromophore was chosen for maximal overlap of its absorbance with the emission spectrum of the fluorophore, resulting in quenching of the nearby fluorophore through resonance energy transfer (Figure 1). The fluorogenic peptide substrate has been demonstrated to be cleaved quantitatively by HIV-PR at a single bond.

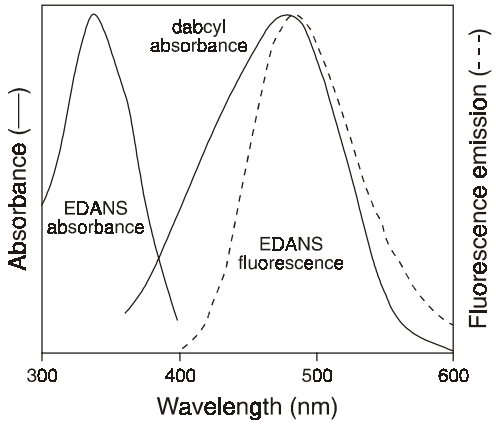

Figure 1. *Spectra showing how the absorption of dabcyl overlaps with the fluorescence of EDANS, quenching the fluorescence through resonance energy transfer.*

Materials

HIV Protease Substrate 1 is provided in a unit size of 1 mg. The substrate can be stored for at least one year, if stored desiccated at 4°C or below, protected from light. A suitable stock concentration for this substrate is 500 µM, which can be prepared by adding 992 µL of high quality anhydrous dimethyl sulfoxide (DMSO) directly to the vial of substrate. This solution should be stable for at least six months when stored desiccated at 4°C, protected from light.

Properties

- Structure: Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(dabcyl)-Arg
- Molecular weight: 2016
- Excitation λ_{max}: 340 nm
- Emission λ_{max}: 490 nm (after cleavage)

HIV Protease Assay Buffer

- 0.1 M sodium acetate
- 1.0 M sodium chloride
- 1.0 mM ethylenediaminetetraacetic acid (EDTA)
- 1.0 mM dithiothreitol (DTT)
- 10% dimethylsulfoxide (DMSO)
- 1 mg/mL bovine serum albumin (BSA)
- pH adjusted to 4.7

General Assay Protocol

1.1 Prepare a solution of 2 µM HIV Protease Substrate 1 in the Assay Buffer.

1.2 Dispense the substrate solution into UV-pass fluorescence cuvettes (1 cm pathlength).

1.3 Set the excitation and emission monochromators of the spectrofluorometer to 340 nm and 490 nm, respectively. To obtain the same kinetic constants as reported, cuvettes should be held at 37°C throughout the assay.

1.4 Begin each assay by adding a small amount (<3% of the final volume) of HIV-PR–containing solution diluted in the assay buffer (see *HIV Protease Assay Buffer*).

1.5 Measure the initial rate of cleavage of fluorogenic substrate by monitoring the increase in fluorescence signal at 490 nm for 5–8 minutes at 37°C.

Notes on Usage of the HIV Protease Substrate 1

2.1 One mg of HIV Protease Substrate 1 is sufficient for approximately 120 assays if HIV-PR reactions are performed with 2 µM substrate in standard 2 mL, 1 cm–pathlength cuvettes. Although 2 µM is well below the K_M of HIV-PR for this substrate, less than 1% of the substrate will be consumed over the 5–8 minutes duration of the measurement at 37°C. The sensitivity of the fluorometric detector will determine the substrate concentration and the lower limit for cuvette size. Given adequate fluorometer sensitivity, 1 mg of substrate is sufficient to assay approximately 1600 samples in 3 × 3 mm 150 µL microcuvettes with 2 µM substrate. However, it may be necessary to increase substrate concentrations above 2 µM to increase the signal if very small volumes of reaction mixture are used, especially when the assay is adapted for use with fluorescence microplate readers. The assay can be used to measure nanomolar concentrations of HIV-PR.

2.2 Despite its mildly inhibitory activity on HIV-PR, the assay buffer contains 10% DMSO to enhance the solubility of the fluorogenic peptide substrate. If sufficiently low substrate concentrations are required, it may be possible to reduce the concentration of DMSO in the assay.

2.3 Reported constants for HIV-1 PR enzyme activity with HIV Protease Substrate 1: $K_M \pm SD = 103 \pm 8$ µM, $V_{max} \pm SD = 164 \pm 7$ nanomoles min^{-1}.

References

1. Science 247, 954 (1990); **2.** Tetrahedron Lett 31, 6493 (1990).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
H-2930	HIV Protease Substrate 1 (Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(dabcyl)-Arg)	1 mg

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