

Product Information

HIV Protease Substrate 1

Catalog Number **H6660**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Synonyms: Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg

Product Description

Formula: $\text{C}_{92}\text{H}_{133}\text{N}_{27}\text{O}_{23}\text{S}$

Molecular Weight: 2,017.3 Da

Excitation λ_{max} : 340 nm

Emission λ_{max} : 490 nm (post-cleavage)

Components

HIV Protease Substrate 1 is a synthetic peptide sequence that contains the cleavage site (Tyr-Pro) for the HIV Protease, as well as two covalently modified amino acids for the detection of cleavage:

- One modification is the addition of the fluorophore EDANS [5-(2-aminoethylamino)-1-naphthalene sulfonate] to the glutamic acid residue.¹
- The other modification is the addition of the acceptor chromophore DABCYL (4'-dimethyl-aminoazobenzene-4-carboxylate) to the lysine residue.¹

The modified amino acids are on opposite sides of the cleavage site. Spatial orientation and overlap of the DABCYL absorbance with the EDANS emission permits resonance energy transfer between the two moieties. Thus quenching of the EDANS fluorescence at 490 nm occurs. However, when the peptide is cleaved by the HIV Protease, the DABCYL group is no longer proximal to the fluorophore. Thus emission at 490 nm can be detected.

Applications of this product include studies of HIV protease kinetics, evaluation of protease inhibitors, and protein structure-function relationships.³⁻⁵

Storage/Stability

The product should be stored at $-20\text{ }^{\circ}\text{C}$, desiccated and protected from light.

Preparation Instructions

A 1 mM stock solution of can be prepared in DMSO.² DMSO stock solutions of this product may be stored desiccated and protected from light at $2-8\text{ }^{\circ}\text{C}$.

Assay Procedure

1. Prepare the following assay buffer:² 0.1 M sodium acetate, 1.0 M sodium chloride, 1.0 mM EDTA, 1.0 mM DTT, 10% DMSO, and 1 mg/mL bovine serum albumin (BSA), pH 4.7.
2. Prepare a 2 μM working solution of the HIV Protease Substrate 1 from the 1 mM stock solution (see Preparation Instructions) by dilution with the assay buffer.
3. Add an appropriate volume of the 2 μM working solution into UV-pass fluorescence cuvettes, which have been pre-warmed to $37\text{ }^{\circ}\text{C}$.
4. The wavelength settings of the spectrofluorometer should be set to 340 nm for excitation and 490 nm for emission detection.
5. Start each assay by adding a small amount of the HIV Protease-containing sample (<3% of the final volume) to the cuvettes.
6. Measure the initial protease cleavage rate by monitoring the increase in emission at 490 nm for 3-5 minutes at $37\text{ }^{\circ}\text{C}$.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

References

1. Matayoshi, E.D. *et al.*, *Science*, **247(4945)**, 954-958 (1990).
2. Lam, T.L. *et al.*, *Life Sci.*, **67(23)**, 2889-2896 (2000).
3. Onaizi, S.A. *et al.*, *Langmuir*, **23(11)**, 6336-6341 (2007).
4. Gaber, R. *et al.*, *Sensors (Basel)*, **13(12)**, 16330-16346 (2013).
5. Cheng, N. *et al.*, *J. Lab. Autom.*, **19(3)**, 297-303 (2014).

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