Revised: 19–August–2001

Renin Substrate 1 (R-2931)

For Fluorometric Measurement of Renin Activity

Quick Facts

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

Abs/Em of product: 340/490 nm

Solvent for stock solution: DMSO

Introduction

Produced in the afferent glomerular arteriole, renin is an aspartic protease that catalyzes the cleavage of the N-terminal decapeptide from angiotensinogen to form angiotensin I. The octapeptide angiotensin II, formed by cleavage of angiotensin I, acts as a potent vasoconstrictor and regulator of Na^+ flux. Although renin activity can be measured by radioimmunoassay techniques, this approach is time consuming and is not useful for kinetic studies.

A fluorometric method that makes use of a synthetic peptide substrate has been shown to be useful for making kinetic measurements [1] and measurements of renin enzyme activity in a fluorescence-based microplate reader.[2] The peptide, which is the normal substrate for renin, has been linked to a fluorophore (5-(aminoethyl)aminonaphthalene sulfonate, EDANS) at one end and to a nonfluorescent chromophore (4′-dimethylaminoazobenzene-4-carboxylate, dabcyl) at the other.[3] The acceptor dabcyl chromophore was chosen for maximal overlap of its absorbance with the emission spectrum of the EDANS fluorophore, resulting in quenching of the nearby EDANS through resonance energy transfer (Figure 1). After cleavage by renin, the product (peptide-EDANS) is brightly fluorescent.

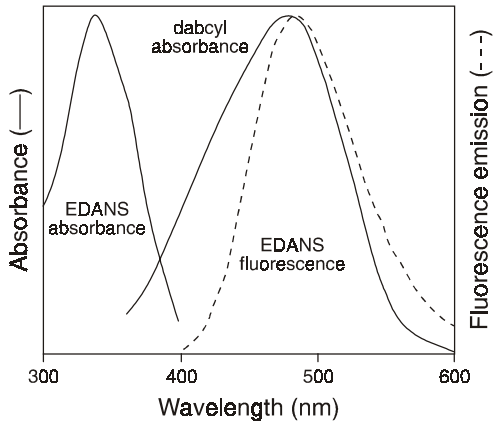

Figure 1. *Spectra showing how the absorption of dabcyl overlaps with the fluorescence of EDANS, quenching the fluorescence through resonance energy transfer.*

Materials

Renin Substrate 1 is provided as a lyophilized powder 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 877 µL of high-quality anhydrous dimethylsulfoxide (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.

Experimental Protocol

One mg of Renin Substrate 1 is sufficient for approximately 100 assays if renin reactions are carried out with 2 µM substrate in standard 2 mL, 1 cm–pathlength cuvettes. 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 1400 samples in 3 × 3 mm 150 µL microcuvettes with 2 µM substrate. However, it may be necessary to increase substrate concentrations above 2 µM 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 renin.

Constants for human recombinant renin activity with Renin Substrate 1: K_M = 3.6 µM, V_{max} = 1.6 F.U. min^{-1}. *See reference 4 for a detailed discussion of determination of these kinetic constants.*

Materials Required but Not Provided

- High quality, anhydrous dimethylsulfoxide (DMSO)
- Renin Substrate 1 assay buffer, which includes 100 mM sodium chloride and 50 mM tris(hydroxymethyl)aminomethane (Tris base) with the pH adjusted to pH 8.0 (note **A**).

General Assay Protocol

1.1 Prepare a solution of 2 µM Renin Substrate 1 in the assay buffer (see *Renin Subtrate 1 Assay Buffer,* above).

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. The 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 renin-containing solution diluted in the 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 min at 37°C.

Notes

[A] The peak of the pH/activity profile of renin using the fluorogenic substrate (pH 8.0) is 2.5 pH units higher than that of renin using an angiotensin assay (pH 5.5).[4]

References

1. J Protein Chem 10, 553 (1991); **2.** Anal Biochem 210, 351 (1993); **3.** Science 247, 954 (1990); **4.** J Protein Chem 9, 663 (1990).

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

Cat #	Product Name	Unit Size
R-2931	Renin Substrate 1 (Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(dabcyl)-Arg)	1 mg

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