CREATIVE BIOMART®
Assay Kit

Protease Fluorometric Assay Kit

Product Information

Cat.No.

Kit-0721

Product Overview

Allows quick and simple measurement of general protease activity in biological samples.

Size

1 kit

Description

Direct fluorescence-based assays for detecting metallo-, serine, acid or sulfhydryl proteases are important in medical, biochemical and cell biology research. Analysis of low levels of protease activity is important in biochemical quality control testing, for analysis of protease inhibitors or cofactors, as well as for basic research application in biology and molecular biology. Several fluorescence-based methods have been developed for detecting protease activity including the fluorescein thiocarbamoyl (FTC)-casein protease assay, in which unhydrolyzed protein must be precipitated with trichloroacetic acid, separated by centrifugation, transferred for measurement and then pH-adjusted to optimize the fluorescence signal. The Fluorescent Protease Assay Kit avoids these time-consuming separation steps by taking advantage of the self-quenching of fluorescein when heavily coupled to protein. This kit uses the conjugated protein, FITC-Casein as a substrate. Casein is a naturally occurring protein in milk that is suitable as a general substrate for a myriad of proteases. Labeled with multiple fluorescent dyes, the substrate exhibits significant fluorescence quenching. Protease-catalyzed hydrolysis releases highly fluorescent-labeled peptides; the accompanying increase in fluorescence is proportional to protease activity and can be conveniently measured in a continuous assay format using a fluorometer equipped with an appropriate (fluorescein) filter set (EX/EM= 490/520 nm). This kit has demonstrated sensitivity of less than 10 mU/mL enzyme. Extensive protease cleavage of the substrate can result in fluorescence increases of greater than 10-fold. In addition to utility for detecting protease contamination of culture media and other experimental samples, the assay can be used to continuously measure the kinetics of a variety of exo-and endopeptidases or to measure the total substrate turnover at a fixed

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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time following addition of the enzyme. Among the enzymes that can be monitored using this method are elastase, chymotrypsin, thermolysin, trypsin, papain, pepsin, cathepsin D and elastase. The kit contains enough substrate for 100 assays and control experiments (96-well microtiterplate, 100 μL reaction volume) and also contains reference standards and a detailed protocol for use. See the references below for more information and applications.

Storage

The substrate and reference standard included in this kit should be stored at -20˚C when not in use. Protect solutions of the substrate and reference standard from light. Substrate solutions are best when prepared fresh, prolonged storage in solution or repeated freeze/thaw cycles may result in denaturation of the protein and/or increased background fluorescence.

Kit Components

A.) Substrate: 5 mg FITC-Casein. Prepare 1mg/mL solution for use in the assay protocol below. B.) Reference Standard: 1 mg Fluorescein-5-thiourea (FTU). Dilute to desired concentration using reaction buffer.C.) Reaction Buffer REAGENTS-QUANTITY-STORAGE Substrate: 1 x 5mg; F, R, L; Reference Standard: 1 x 1mg; F, R, L; Reaction Buffer: 1 x 30mL; CNotes: F=store at or below -20°C; C=store cold (4°C); L=light sensitive; T=avoid repeat freeze/thaw; R=read protocol;instructions carefully prior to use.