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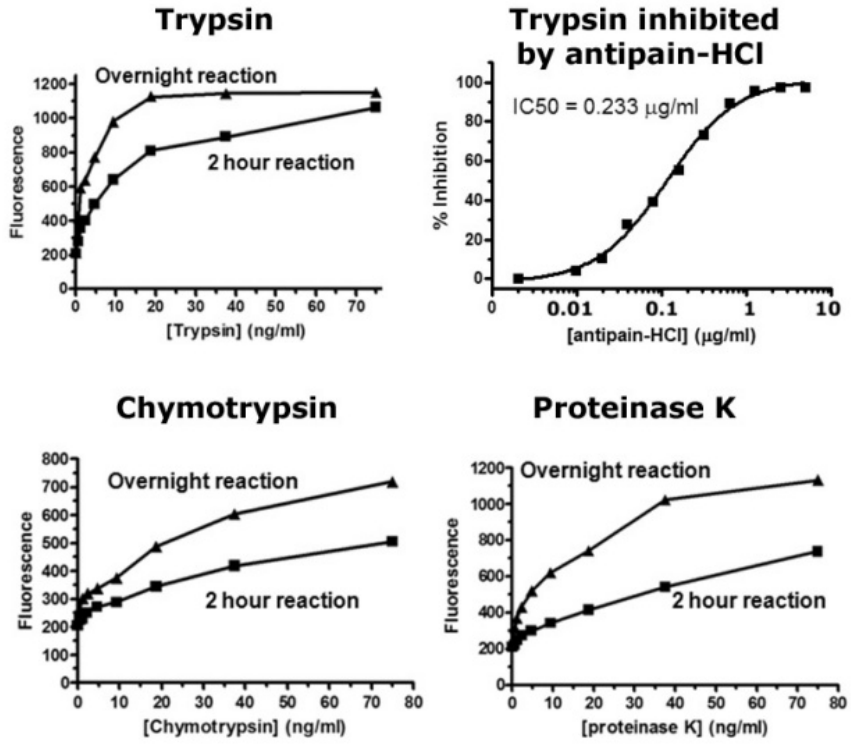
INSTRUCTIONS

ProFoldin
Ultra-sensitive High-throughput Protease Assay Kit

Catalog Number UPA1000

Introduction

Protease contamination even in a trace amount can degrade proteins or peptides during purification, storage or crystallization. Protein or peptide drugs should be protease-free. Proteases are also important drug targets. The Ultra-sensitive High-throughput Protease Assay Kit is useful to detect protease contamination of various protein preparations and to screen protease inhibitors in a 96-well or 384-weel plate assay format. The assay is based on the principle that digestion of the fluorescence-labeled protein substrate results in dramatic increase of the fluorescence signal. The sensitivity of the assay of trypsin, chymotrypsin and proteinase K is below 5 ng/ml or 0.2 ng per assay well for the standard 384-well plate assay.



The **Ultra-sensitive High-throughput Protease Assay Kit** (Catalog number UPA1000) includes the 5 ml of 10 x assay buffer and 50 ul of 1000 x substrate for 1000 assays of proteases in a 384-well plate format.

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Assay Protocol

1. Reagent preparation

Thaw the 1000 x protease substrate from the frozen stock. Mix the stock solution well by shaking or vortex. Dilute the 1000 x protease substrate 100-fold with water to make the 10 x protease substrate.

2. Assay

In a standard black 384-well plate (Corning plate 3571, for example), mix 5 µl of 10 x buffer, 40 µl of the protein sample and 5 µl of 10 x protease substrate. Incubate the reaction mixture at 37°C for 2 hours. Measure the fluorescence intensity at 535 nm (excitation at 485 nm).

Note: It is a continuous assay. The fluorescence signal can be measured at different time points. A longer incubation including overnight or days allows to detect a lower concentration of protease.

Assay Protocol for enzyme inhibition

Enzyme inhibition IC_{50} can be measured using the 384-well or 96-well plate assay format. Typically 50 x stock solutions with a 2-fold serial dilution in water or DMSO are prepared. For 96-well plate assays, 4 µl of the 50 x inhibitor is mixed with 176 µl of the assay reaction mixture composed of the buffer and enzyme for 5 min. Then 20 µl of 10 x substrate is added. At the end of the reaction, the fluorescence intensity is measured.

More information of drug targets and enzyme assays

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.