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Amplite™ Universal Fluorimetric Protease Activity Assay Kit *Green Fluorescence*

Catalog number: 13500
Unit size: 500 Tests

Component	Storage	Amount
Component A: Protease Substrate	Freeze (<-15 °C), Avoid Light	1 vial (300 µL)
Component B: Trypsin	Freeze (<-15 °C), Avoid Light	1 vial (100 µL, 5 U/µL)
Component C: 2X Assay Buffer	Refrigerate (2-4 °C), Avoid Light	1 bottle (30 mL)

OVERVIEW

Monitoring of various protease activities has become a routine task for many biological laboratories. Our Amplite™ Universal Fluorimetric Protease Activity Assay Kits are an ideal choice for performing routine assays necessary during the isolation of proteases, or for identifying the presence of contaminating proteases in protein samples. The kits use fluorescent casein conjugates that are proven to be a generic substrate for a broad spectrum of proteases. In the intact substrate, casein is heavily labeled with a fluorescent dye, resulting in significant fluorescence quenching. Protease-catalyzed hydrolysis relieves its quenching effect, yielding brightly fluorescent dye-labeled short peptides. The increase in fluorescence intensity is directly proportional to protease activity. The kits provide all the essential components with an optimized "mix & read" protocol that can be easily automated to HTS instruments.

AT A GLANCE

Protocol summary
Measuring protease activity in test samples (Protocol A)

1. Prepare protease substrate solution (50 µL)
2. Add substrate control, positive control or test samples (50 µL)
3. Skip incubation for kinetic reading or incubate for 30 to 60 minutes for end point reading
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Protocol summary
Screening protease inhibitors using a purified enzyme (Protocol B)

1. Prepare protease substrate solution (10 µL)
2. Add substrate control, positive control, vehicle control or test samples (90 µL)
3. Skip incubation for kinetic reading or incubate for 30 to 60 minutes for end point reading
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Thaw all the kit components at room temperature before starting the experiment. **Please choose Protocol A or Protocol B according to your needs.**

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

For Protocol A

1. Protease substrate solution:
Dilute Protease Substrate (Component A) at 1:100 in 2X assay buffer (Component C). Use 50 µL of protease substrate solution per assay in a 96-well plate.

Note The 2X Assay Buffer (Component C) is designed for detecting the activity of chymotrypsin, trypsin, thermolysin, proteinase K, protease XIV, and human leukocyte elastase. For other proteases, please refer to Table 1 below for the appropriate assay buffer formula.

2. Trypsin dilution:
Dilute Trypsin (5 U/µL, Component B) at 1:50 in de-ionized water to get a concentration of 0.1 U/µL.

For Protocol B

1. Assay buffer (1X):
Add 5 mL de-ionized water into 5 mL of 2X Assay Buffer (Component C).

2. Protease substrate solution (for Protocol B):
Dilute Protease Substrate (Component A) at 1:20 in 1X assay buffer. Use 10 µL/well of protease substrate solution for a 96-well plate.

Note The 2X assay buffer (Component C) is designed for detecting the activity of chymotrypsin, trypsin, thermolysin, proteinase K, protease XIV, and human leukocyte elastase. For other proteases, please refer to Table 1 below for the appropriate assay buffer formula.

3. Protease dilution:
Dilute the protease in 1X assay buffer to a concentration of 500 - 1000 nM. Each well will need 10 µL of protease dilution. Prepare an appropriate amount for all the test samples and extra for the positive control and vehicle control wells.

Table 1. Assay buffer formulas for proteases. For protocol A, 2X assay buffer is needed. For protocol B, 1X assay buffer is needed.

Protease	1X or 2X Assay Buffer
Cathepsin D	20 mM Sodium Citrate, pH 3.0
Papain	20 mM sodium acetate, 20 mM cysteine, 2 mM EDTA, pH 6.5
PAE	20 mM sodium phosphate, pH 8.0
Pepsin	10 mM HCl, pH 2.0
Porcine pancreas elastase	10 mM Tris-HCl, pH 8.8
Subtilisin	20 mM potassium phosphate buffer, pH 7.6, 150 mM NaCl

SAMPLE EXPERIMENTAL PROTOCOL

Protocol A: Measure protease activity in test samples

Table 2A. Layout of the substrate control, positive control, and test samples in a 96-well microplate. SC=Substrate Control, PC =Positive Control, TS=Test Samples.

SC	SC	...	...
PC	PC	...	...
TS	TS		
...	...		

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Table 3A. Reagent composition for each well. If less than 50 µL of protease-containing biological sample is used, add ddH_2O to make a total volume of 50 µL.

Well	Volume	Reagent
SC	50 µL	De-ionized water
PC	50 µL	Typsin dilution
TS	50 µL	Protease-containing samples

1. Add 50 µL of protease substrate solution (Protocol A) to all the wells in the assay plate. Mix the reagents well.

2. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 490/525 nm.

   For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30 minutes.

   For end-point reading: Incubate the reaction at a desired temperature for 30 to 60 minutes, protected from light. Then measure the fluorescence intensity.

Protocol B: Screening protease inhibitors using a purified enzyme

Table 2B. Layout of the samples in a 96-well microplate. SC=Substrate Control, PC= Positive Control, VC=Vehicle Control, TS=Test Samples. It's recommended to test at least three different concentrations of each test compound. All the test samples should be done in duplicates or triplicates.

SC	SC	...	...
PC	PC	...	...
VC	VC		
TS	TS		
...	...		

Table 3B. Reagent composition for each well. For each volume of test compound added into a well, the same volume of solvent used to deliver test compound needs to be checked for the effect of vehicle on the activity of protease.

Well	Volume	Reagent
SC	90 µL	Assay Buffer (1X) (90 µL)
PC	90 µL	Assay Buffer (1X) (80 µL) Protease dilution (10 µL)
VC	90 µL	Vehicle (X µL) Assay Buffer (80 - X µL) Protease dilution (10 µL)
TS	90 µL	Test compound (X µL) Assay Buffer (1X) (80 - X µL) Protease dilution (10 µL)

1. Add 10 µL of protease substrate solution (Protocol B) into the wells of positive control (PC), vehicle control (VC), and test sample (TS). Mix the reagents well.

2. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 490 /525 nm.

   For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30 minutes.

   For end-point reading: Incubate the reaction at a desired temperature for 30 to 60 minutes, protected from light. Then measure the fluorescence intensity.

EXAMPLE DATA ANALYSIS AND FIGURES

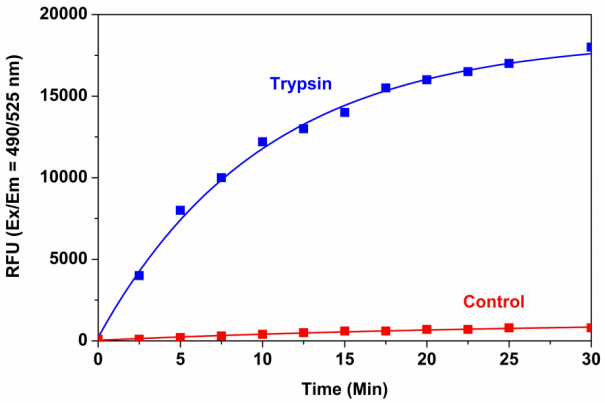
The fluorescence in the substrate control wells is used as a control, and is subtracted from the values for other wells with the enzymatic reactions.

Plot data as relative fluorescence unit (RFU) versus time for each sample (as shown in Figure 1).

Determine the range of initial time points during which the reaction is linear. 10 - 15% conversion appears to be the optimal range.

Obtain the initial reaction velocity (V_0) in RFU/min. Determine the slope of the linear portion of the data plot.

A variety of data analyses can be done, e.g., determining inhibition %, IC_{50}, K_m, K_i, etc.



Trypsin protease activity was analyzed by Amplite™ Universal Fluorimetric Protease Activity Assay Kit. Protease substrate was incubated with 1 unit trypsin in the kit assay buffer. The control wells had protease substrate only (without trypsin). The fluorescence signal was measured starting from time 0 when trypsin was added. Samples were done in triplicates.

DISCLAIMER