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

Ordering Information:
Product number: 13500 (500 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in -20°C avoid light. *Components C and D can be stored at 4 °C for convenience.*

Introduction

Protease assays are widely used for the investigation of protease inhibitors and detection of protease activities. Monitoring of various protease activities has become a routine task for many biological laboratories. Our Amplite™ Universal Fluorimetric Protease Activity Assay Kits is 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 (e.g. trypsin, chymotrypsin, thermolysin,proteinase K, protease XIV, and elastase). 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 assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by fluorescence microplate reader with Ex/Em = 490 nm/520 nm.

Kit Features and Benefits

Convenient Format :	Kit includes all the key assay components.
Optimized Performance:	Optimal conditions for the detection of generic protease activity.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Protease substrate	1 vial (300 µL), protect from light
Component B: Trypsin	1 vial (100 µL, 5 U/µL)
Component C: 2X Assay buffer	1 bottle (30 mL)

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Assay Protocols for one 96-well plate

Please choose Protocol A or B based on your needs.

Protocol A: Measure protease activity in test samples

Brief Summary

Prepare protease substrate solution (50 µL) → Add substrate control, positive control or test samples (50 µL) → Incubate for 0 min (for kinetic reading) or 30 min-1 h (for end point reading) → Read fluorescence at Ex 490 nm/Em 520nm

Thaw all the kit components to room temperature before starting your experiment.

1. Prepare working solutions:

1.1 Make protease substrate solution: Dilute protease substrate (Component A) at 1:100 in 2X assay buffer (Component C). You will need 50 µL of protease substrate solution per assay in 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 **Appendix I** for the appropriate assay buffer formula.*

1.2 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.

2. Add reagents prepared in step 1 into a 96-well microplate according to Table 1 and Table 2.

Table 1. Layout of the substrate control, positive control, and test samples in a 96-well microplate:

SC	SC										
PC	PC										
TS	TS										
….	….										

Note: SC=substrate control, PC positive control, TS=test samples.

Table 2. Reagent composition for each well:

Substrate Control	Positive Control	Test Sample
De-ionized water: 50 µL	Typsin dilution: 50 µL	Protease-containing sample: 50 µL
Total volume: 50 µL	Total volume: 50 µL	Total volume: 50 µL

Note: If less than 50 µL of protease-containing biological sample is used, add ddH2O to a total volume of 50 µL.

3. Run the enzymatic reaction.

3.1 Add 50 µL protease substrate solution to all the wells in the assay plate. Mix the reagents well.

3.2 Monitor the fluorescence increase with 490 nm excitation and 520 nm emission by using a fluorescence plate reader.
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 the desired temperature for 30 to 60 minutes, and protected from light. Then measure the fluorescence intensity.

4. Data analysis: Refer to **Run Data Analysis** section**.**

Protocol B: Screening protease inhibitors using a purified enzyme

Brief Summary

Prepare protease substrate solution (50 µL) → Add substrate control, positive control, vehicle control or test samples (50 µL) → Incubate for 0 min (for kinetic reading) or 30 min-1 h (for end point reading) → Read fluorescence at Ex 490 nm/Em 520nm

1. Prepare working solutions:

1.1 Make 1X assay buffer: Add 5 mL de-ionized water to 5 mL of 2X assay buffer (Component C).

1.2 Make protease substrate solution: Dilute protease substrate (Component A) at 1: 20 in 1X assay buffer (from step 1.1). You will need 10 µL of protease substrate solution per assay in 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 ***Appendix I*** *for the appropriate assay buffer formula.*

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

2. Add reagents prepared in step 1 into a 96-well microplate according to Table 1 and Table 2.

Table 1. Layout of the samples in a 96-well microplate:

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

Note1: SC=substrate control, PC positive control, VC=vehicle control, TS=test samples.
Note 2: We recommend testing at least three different concentrations of each test compound. All the test samples should be done in duplicates or triplicates.

Table 2. Reagent composition for each well:

Substrate Control	**Positive Control**	**Vehicle Control**	**Test Sample**
1X assay buffer: 90 µL	1X assay buffer: 90µL Protease dilution: 10 µL	Vehecle*: X µL 1X assay buffer: (80-X) µL Protease dilution: 10 µL	Test compound: X µL 1X assay buffer: (80-X) µL Protease dilution: 10 µL
Total volume: 90 µL	Total volume: 90 µL	Total volume: 90 µL	Total volume: 90 µL

*Note: *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.*

3. Run the enzymatic reaction.

3.1 Add 10 µL protease substrate solution to the positive control (PC), vehicle control (VC), and test sample (TS) wells. Mix the reagents well.

3.2 Monitor the fluorescence intensity with 490 nm excitation and 520 nm emission by using a fluorescence plate reader.
For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 3 minutes for 30 minutes.
For end-point reading: Incubate the reaction at the desired temperature for 30 to 60 minutes, and protected from light. Then measure the fluorescence intensity.

4. Data analysis: Refer to **Run Data Analysis** section**.**

Run Data Analysis

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 (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 (Vo) 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.

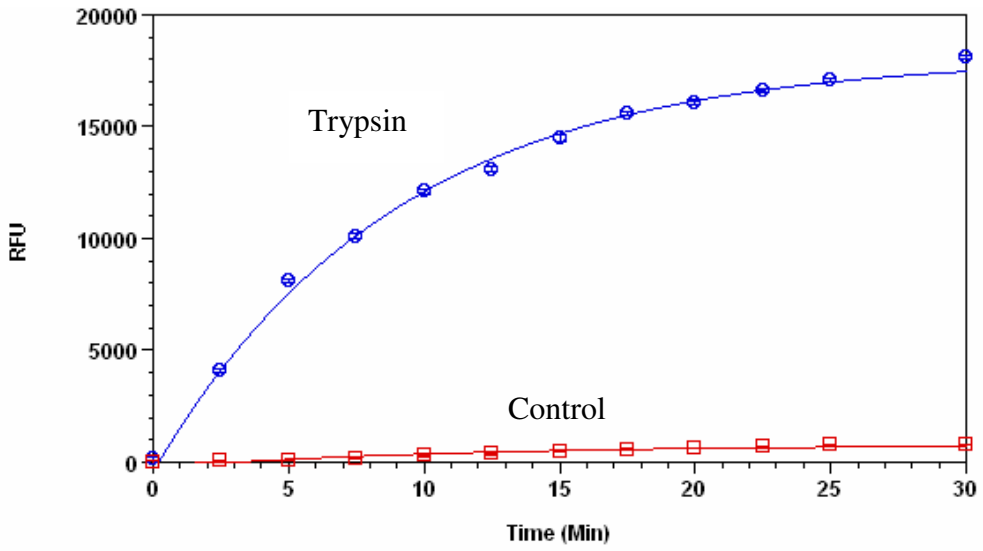

Figure 1. Proteolytic cleavage of Amplite™ Universal Fluorimetric Protease substrate by trypsin. Protease substrate was cleaved by 1 unit trypsin in assay buffer. The control wells had protease substrate only without trypsin. The fluorescence signal was measured starting from Time 0, when trypsin was added, by using a BMG LabTech NOVOStar microplate reader with a filter set of Ex/Em=485 nm/520 nm. Samples were done in triplicates.

Appendix I:

Protease	1X 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 Tris-HCl, pH 8.8
Porcine pancreas elastase	10 mM HCl, pH 2.0
Subtilisin	20 mM potassium phosphate buffer, pH 7.6, 150 mM NaCl

* For protocol A, 2X assay buffer is needed. For protocol B, 1X assay buffer is needed.

References:

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