Protease Activity Assay Kit

Item No. 701410

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Customer Service 800.364.9897
Technical Support 888.526.5351
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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity	Storage
701411	Protease Assay Buffer	1 vial/20 ml	-20°C
701412	Protease Substrate	2 vials/lyophilized	-20°C
701413	Protease Positive Control	2 vials/lyophilized	-20°C
400017	96-Well Solid Plate (black)	1 plate	RT
400023	Foil Plate Covers	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence or fluorescence polarization using fluorescein as the fluorophore
2. Adjustable pipettes and a multichannel or repeating pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

About This Assay

Cayman's Protease Activity Assay Kit provides a convenient method for determining the activity of proteases in samples. Proteolytic digestion of the FITC-casein substrate can be monitored by changes in either fluorescence polarization (FP) or total fluorescence.[1] Fluorescence is the ability of a molecule to absorb the energy of an incoming (excitation) photon and then re-emit this energy as a new, slightly less energetic (emission) photon. A small fluorescent molecule will rotate appreciably during the very small interval of time between absorption of a photon and emission of the fluorescence photon. If the excitation light is polarized, this rotation will result in randomization of the plane of the emitted light. Thus, small fluorescent molecules depolarize an excitation pulse of polarized light. Large fluorescent molecules, like FITC-casein, do not rotate as appreciably in the same small interval of time. They will, therefore, emit light that retains some of the polarization of the polarized excitation light. This polarization is quantified as millipolarization units, or mP. In this assay, the total FP decreases as the FITC-casein is digested into smaller, quicker rotating fluorescein-labeled fragments.

If measuring FP is not available, the change in total fluorescence can also be measured to determine protease activity. The conjugation of FITC to casein results in moderate quenching of the total fluorescence. This fluorescence increases as the FITC-casein substrate is digested into smaller fluorescein-labeled fragments. Both assay formats use an excitation wavelength of 480-495 nm and an emission wavelength of 515-525 nm.

Figure 1. Changes in fluorescence are observed after digestion of FITC-casein by proteases

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Protease Assay Buffer - (Item No. 701411)**

   This vial contains 20 ml of 25 mM Tris, pH 7.2, containing 150 mM sodium chloride. Once thawed, this buffer is ready to use, and remaining buffer can be stored at -20°C for up to six months.

2. **Protease Substrate - (Item No. 701412)**

   Each vial contains lyophilized FITC-casein. Reconstitute the contents of the vial with 6 ml of Protease Assay Buffer. One vial of FITC-casein substrate should be sufficient substrate to assay 60 wells. Use the additional vial if assaying the entire plate. *NOTE: Avoid exposing the FITC-casein to intense light*. Remaining Protease Substrate can be stored at -20°C for up to six months.

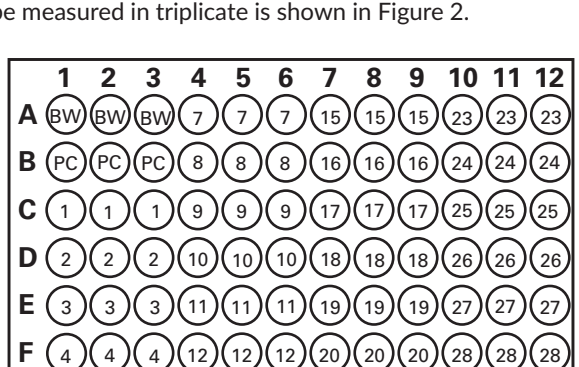
3. **Protease Positive Control - (Item No. 701413)**

   Each vial contains lyophilized trypsin. Reconstitute the contents of a vial with 500 µl of Protease Assay Buffer, store on ice and use within two hours. Each vial should be sufficient to run four positive control wells.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is recommended to have three wells designated as Protease Positive Control and three wells designated as Background. It is suggested that the contents of each well are recorded on the template sheet provided on page 14. A typical layout of samples to be measured in triplicate is shown in Figure 2.



BW - Background Wells
PC - Positive Control Wells
1-30 - Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all the wells.
- All reagents, except the Protease Positive Control (Item No. 701413), must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in triplicate, but it is the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence or FP with an excitation wavelength of 480-495 nm and an emission wavelength of 515-525 nm.

Performing the Assay

1. **Positive Control Wells -** add 100 µl of Protease Positive Control (Item No. 701413) to three wells.
2. **Sample Wells -** add 100 µl of sample to at least three wells.
3. **Background Wells -** add 100 µl of Protease Assay Buffer to three wells.

Well	Protease Assay Buffer (µl)	Protease Positive Control (µl)	Sample (µl)
Positive Control wells	-	100	-
Sample wells	-	-	100
Background wells	100	-	-

Table 1. Pipetting summary

4. Initiate the reactions by adding 100 µl of Protease Substrate to all the wells being used.
5. Cover the plate with the foil plate cover and incubate for twenty minutes at room temperature on an orbital shaker.
6. Remove the plate cover, and measure the fluorescence or FP with an excitation wavelength of 480–495 nm and an emission wavelength of 515–525 nm.

ANALYSIS

Calculations

1. FP of a molecule is defined as:

$$\text{Polarization (mP)} = 1{,}000 \times \frac{(I_{\parallel} - I_{\perp} \times G)}{(I_{\parallel} + I_{\perp} \times G)}$$

where:
$I_{\parallel}$ = Fluorescence intensity parallel to the excitation plane
$I_{\perp}$ = Fluorescence intensity perpendicular to the excitation plane
G = Instrument-specific correction factor

2. Determine the average fluorescence or FP of each sample.
3. Subtract the average fluorescence of the background sample from itself and all other values. This is the corrected fluorescence.
4. Plot the corrected fluorescence or non-corrected mP *versus* protease concentration. An example is shown in Figure 3, see page 12.

Performance Characteristics

Sample Data:

The data shown here is an example of FP or total fluorescence data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

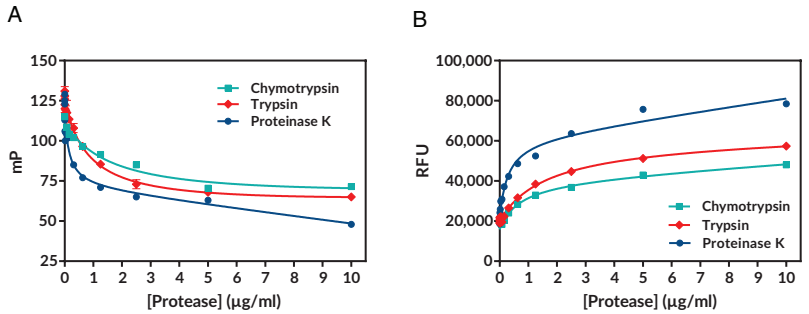


Figure 3. Digestion of FITC-casein by trypsin, chymotrypsin, and proteinase K. (A) Fluorescence polarization (B) Total fluorescence.

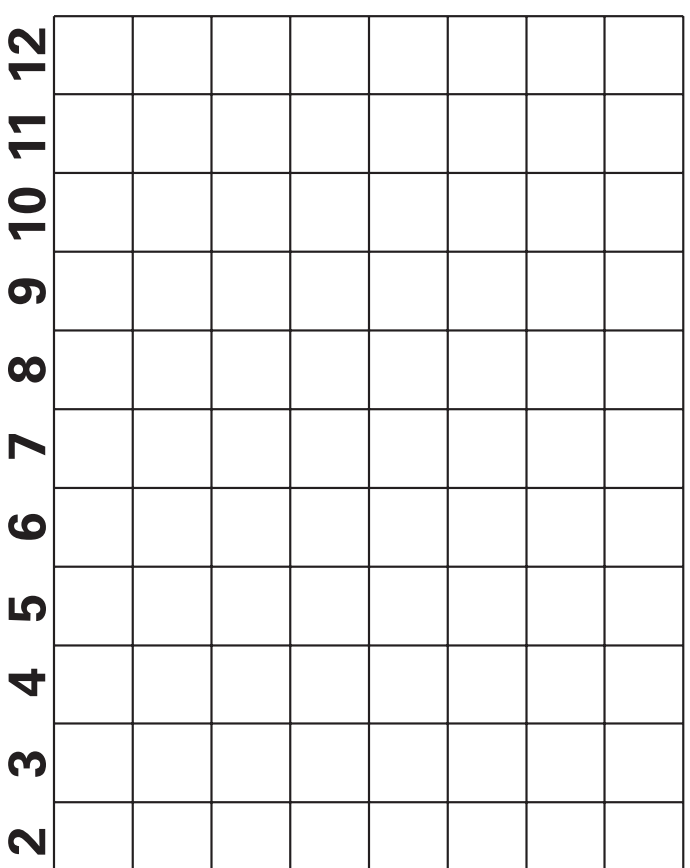
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the well(s) B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence was detected above background	Enzyme or substrate was not added to the well(s)	Make sure to add all of the components to the well(s) or increase the incubation time before reading the plate
The plate reader exhibited 'MAX' values for the well(s)	The *gain* setting is too high	Reduce the *gain* and re-read
High background	Dilution error	Check the dilution of each component

Reference

1. Twining, S.S. Fluorescein isothiocyanate-labeled casein assay for proteolytic enzymes. *Anal. Biochem.* **143(1)**, 30-34 (1984).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.