PROMETHEUS™
Protein Biology Products

Instruction Manual

Protease Assay Kit **Catalog No.: 18-439**

INTRODUCTION

Proteases are enzymes, naturally present in all living cells and tissues, involved in a multitude of physiological reactions that includes digestions of proteins in the food to highly regulated cascades. Proteases can either break specific peptide bonds, depending on the amino acid sequence of a protein or completely breakdown peptides to amino acids. In certain physiological conditions, protease activity can abolish a protein's function or an activation of a function. For studying proteins, tissues and cells are lysed, which release proteases in the sample and their activities can be measured using a protease assay. Our protease assay kit is a sensitive, universal protease assay kit, which is designed for the quantitative determination of the proteases present in the protein sample and can detect approximately 10ng/ml of proteases in 2 to 24 hours incubation. The kit is supplied with a protease substrate: casein labeled with resorufin and with protease activities in the test sample, resorufin-labeled peptides are released, that cannot be precipitated by the precipitating agent. The concentration of these resorufin-labeled peptides in the supernatant is directly proportional to the proteolytic activity present in the test sample, which can be measured spectrophotometrically at 574nm.

Items Supplied:

Item Name	Cat. No. 18-439	Storage Condition*
Incubation Buffer	6 ml	4°C
Stop Solution	6 ml	4°C
Assay Buffer	15 ml	4°C
Casein-Resorufin Substrate	150 µl	-20°C
Protease (+) Control: Trypsin	2 x 20 µg	-20°C

**The kit is shipped at ambient temperature and upon receipt, store the kit components as marked. The kit components are stable for 12 months, if stored and used as recommended.*

Preparation Before Use

Dissolve the supplied 20 µg Trypsin/vial in 250µl **1mM HCl** *(Take 4.1 µl of Conc. HCl to 50 ml in DI Water, HANDLE IT VERY CAREFULLY)* or **50mM Acetic acid** (*Take 143 µl of Glacial Acetic acid to 50 ml with DI Water, HANDLE IT VERY CAREFULLY*) to prepare **stock** trypsin with concentration 80ng/µl. Serially dilute the stock trypsin (80ng/µl), 1:1 with Incubation Buffer to get trypsin dilutions from 40ng/µl to 1.25ng/µl for preparing Trypsin Standard curve.

NOTE: *The supplied Trypsin is stable for two years, if stored **unopened** at -20°C. An acidic, reconstituted trypsin solution (pH ~ 3.0) can be stored at -20°C for 2 weeks or at –70 °C for ~ 4 weeks, and is stable for at least 2-3 freeze-thaw cycles.*

Choice of Assay Conditions

The Protease Assay Kit is supplied with an incubation buffer, pH 7.8. This pH is recommended for detecting the broadest range of physiological protease activities (i.e. pH 7.4-7.8). Many enzymes, however, have quite different pH optima. For most physiological applications, the incubation buffer (pH 7.8) provided will yield useful protease activity information. However, if a specific enzyme with a unique pH optimum is a suspected contaminant, or if a pH activity curves are desired, we suggest preparing pH-specific buffers.

Positive Control

TPCK treated proteomic grade Trypsin is supplied as a protease positive (+) control in the kit, however, it's not necessary for the interpretation of assay results, as a qualitative positive control for the assay. We recommend that a single positive control sample consisting of 10 µl protease positive control be included with each assay run.

Standard Curve:

When quantitating the protease activity, a standard curve may be generated using the supplied Sequencing Grade Trypsin for relative comparison of overall protease activity in test samples. However, for specific protease activity, preparation of standard curve with specific protease can be achieved by using the supplied universal substrate and other components of the kit.

Assay Protocol

Our protease assay has been designed for 96 well microtiter plate and can be first performed in microcentrifuge tubes, then the final reaction product transferred to a 96-well titer plates for measuring the absorbance at 574, due to centrifugation step involved and if the centrifuge adaptor for 96-well plate is not available.

1. Set up the reaction for test sample for protease assay and trypsin standard in duplicate as in the table below:

Items	**Blank**	**Trypsin Standard**	**Test Sample**
Casein-Resorufin Substrate	2.5 µl	2.5 µl	2.5 µl
Sample	N/A	10 µl	1-45 µl
Incubation Buffer	47.5 µl	37.5 µl	Final volume to 50 µl as per the Test Sample volume added
Close the tubes or seal the plates and incubate at 37°C for 2 hours to 24 hours* (**Note:** longer incubation time needed for slow acting proteases).			

**The sensitivity of the assay can typically be improved 5-10 folds by increasing the incubation time from 3 to 24 h, however, actual increase in sensitivity will depend on the stability of the protease to auto-degradation and thermal breakdown.*

2. After the incubation, add 50 µl Stop Solution, mix the contents and incubate the tubes/plate again at 37°C for 10 minutes.
3. Centrifuge the tubes at 12,000 x g for 5 minutes and for 96 well titer plate at 4,000 x g for 15 minutes.
4. Carefully transfer 80 µl supernatant to clean tubes or wells without disturbing the pellet.
5. Add 120 µl Assay Buffer in each tube or well and mix it, which will instantly develop a pink color.
6. Read the absorbance of the color developed at 574 nm against the blank. The intensity of the color developed in each tube is proportional to the protease activity, which may be assessed using the Trypsin standard calibration curve.

Choice of Standards

Detection of protease activity using this kit does not require that a standard curve be generated. In the case of crude samples containing one or more unknown proteases, a standard curve would have little significance. Protease activity can be related in terms of changes in absorbance at 574 nm per minute. This value can be normalized to the assay volume or mass (total protein concentration) of the sample. The following guidelines are recommended for the preparation and storage of a standard stock protease solution:

1. Dilute the working concentration just before use.
2. The pH of the stock should be as far away as possible from the pH optimum of the protease to minimize auto-degradation. For instance, trypsin is commonly stored at pH 3.0 in dilute HCl solutions. The pH should be adjusted during the preparation of working solutions.
3. Even taking these precautions, enzyme stability may be compromised with time; we suggest customers empirically determine the stability of their protease standards.

RELATED PRODUCTS:

1. **Protease Inhibitor Cocktails (Cat. No. 18-420, 18-425, 18-427, 18-430, 18-433 and 18-436)**
   For inhibiting protease activity in the protein extracts: General, Bacterial, Mammalian, Plant, Recombinant and Yeast/Fungi.
2. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409, 18-411**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples.
3. **RIPA Lysis Buffer (18-415, 18-416 and 18-417:**
   For extracting proteins from different species samples.
4. **Protein Loading Buffer [2X], Cat. No. 20-309:**
   Non-reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.
5. **Protein Loading Buffer [2X], Cat. No. 20-310:**
   Reducing ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.

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