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A Geno Technology, Inc. (USA) brand name

ProteSEEKER™

INTRODUCTION

ProteSEEKER™ is a simple kit designed to identify the specific type of proteases present in your cell or tissue lysates, and allows you to determine the minimal number of individual protease inhibitors required for your sample. For researchers using commercial protease inhibitor, a common problem is that some biological samples have elevated levels of proteases compared to the established norms. In this scenario, the general protease inhibitor cocktails are inefficient resulting in degradation of your proteins. One option is to increase the amount of general protease inhibitor cocktail used, which is very cost ineffective or to identify the elevated protease and then supplement your extraction or lysis buffer with extra protease inhibitors specific for that protease.

ProteSEEKER™ consists of eleven ready to use individual protease inhibitors (see below) at a 100X concentration and a sensitive Protease Screening Kit. The concentration of the protease inhibitors are adjusted to inhibit >90% activity of individual protease in crude tissue extracts at 1X concentration. The principle of *ProteSEEKER™* is that small quantities of your samples are supplemented with each one of the 11 inhibitors supplied. Each sample is screened with the Protease Screening kit and the results used to determine which protease species is present in your sample.

The Protease Screening kit is based on a ready to use dye-labeled protein, the protease substrate, which is digested by proteases to release dye-labeled peptides. The absorbance of which is measured at 574nm for determination of protease activity.

ITEM(S) SUPPLIED CAT # 786-325

Inhibitor	Inhibits	Size
ALLN; N-Acetyl-Leu-Leu-Nle-CHO (Calpain Inhibitor I)	Cell-permeable, peptide aldehyde inhibitor of Calpain and other neutral cysteine proteases	25µl
Antipain-dihydrochloride	Papain, trypsin & plasmin	25µl
Aprotinin	Serine proteases (plasmin, kallikrein, trypsin, chymotrypsin)	25µl
Bestatin	Amino-peptidases & other exopeptidases (not carboxypeptidases)	25µl
Chymostatin	α, β, γ, δ chymotrypsin	25µl
E-64 (N-(N-(L-3 Trans-carboxirane-2-carbonyl)-L-leucyl)-agmatine)	Papain & other cysteine proteases (cathepsin B & L)	25µl
EDTA-Na ₂	Metalloproteases	25µl
Leupeptin	Serine & Cysteine proteases (Plasmin, Trypsin, Papain & Cathepsin B)	25µl
Pepstatin	Aspartic proteases (pepsin, renin, cathepsin D, chymosin)	25µl
AEBSF (4-(2-Aminoethyl)-benzenesulfonyl-fluoride, hydrochloride)	Serine proteases (trypsin, chymotrypsin, plasmin, plasma kallikrein, & thrombin)	25µl
Phosphoramidon	Thermolysin, collagenase & other metalloendoproteinases	25µl
PMSF (Phenylmethylsulfonyl fluoride)	Serine proteases (chymotrypsin, trypsin & thrombin), cysteine proteases (papain)	25µl
Protease Substrate (Lyophilized; 150µl)	N/A	1 vial
Incubation Buffer	N/A	5ml
Precipitation Agent	N/A	5ml
Assay Buffer	N/A	6ml



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STORAGE CONDITIONS

The kit is shipped at ambient temp. Upon arrival, store it at 4°C and the kit components are stable for 1 year.

ITEMS NEEDED BUT NOT SUPPLIED

Microtiter plates, microcentrifuge tubes, plate reader, spectrophotometer and centrifuge.

NOTE: The assay is designed for microtiter plate. The use of microtiter plate requires use of a centrifuge adapted for microtiter plates. Alternatively, the assay may be performed in microcentrifuge tubes and the final reaction product is transferred to microtiter plates for the measurement of optical density.

PREPARATION BEFORE USE:

Protease Substrate: Reconstitute the supplied Protease Substrate by adding 150µl water into the vial, mix it to dissolve completely. After reconstitution, store the substrate at -20°C.

Inhibitors: Allow the solutions to warm to room temperature, vortex the vial for 15-20 seconds and then briefly centrifuge before use. Add appropriate volume of 100X inhibitor solution(s) in the samples to be tested to give a final 1X concentration.

PROTOCOL

1. In a 96 well plate, or microcentrifuge tube, aliquot in the following reagents.

NOTE: All eleven protease inhibitors may be screened or a select few may be used.

NOTE: Your sample may need to be diluted depending on the concentration of proteases in your test sample.

Items	Blank	Control	Test
Test Sample	None	40µl	40µl
Inhibitor, 100X	None	None	0.5µl
Protease Substrate Solution	2.5µl	2.5µl	2.5µl
Incubation Buffer	47.5µl	7.5µl	7µl

2. Seal the plate or tube and incubate at 37°C for 2-3 hours.

NOTE: For slow acting proteases, incubation time may be extended up to 24 hours.

3. After incubation, add 50µl Precipitation Agent, mix the contents and incubate again at 37°C for 10 minutes.

4. Centrifuge the microtiter plate at 2-4,000xg for 15 minutes or tubes at 12,000xg for 5 minutes.

5. Transfer the supernatant to clean tubes. Add 120µl Assay Buffer to each tube and mix. A pink color will develop instantly. The intensity of the color developed in each tube is proportional to protease activity. Read the reaction color at 574nm against the blank.

NOTE: If the assay is performed in microcentrifuge tube, transfer the reaction product to a microtiter plate and read the color at 574nm against the blank.

CALCULATION OF PERCENT INHIBITION

Inhibition of protease is dependent on the nature and concentration of both inhibitors and protease activity. Changing either the test sample size (i.e. protease concentration) or the volume of inhibitor (i.e. inhibitor concentration) into the reaction mixture may change the percent inhibition. For increasing percent inhibition, concentration of inhibitor in the reaction mixture may be increased. Calculate the percent inhibition of protease activity as follows:

$$\% \text{ Protease activity present} = \frac{\text{OD Test} \times 100}{\text{OD Control}}$$

$$\% \text{ Protease activity Inhibition} = 100 - \% \text{ protease activity present}$$

Use the result and the table on page 1 to determine the protease species present in your sample. Individual protease inhibitors are available in larger quantities for preparative scale use.

RELATED PRODUCTS

1. **Protease Arrest (Cat# 786-108):** A cocktail of protease inhibitors for use during protein extraction and purification. Protease Arrest inhibits a broad spectrum of serine, cysteine and metalloproteases as well as calpains.

2. **Focus Protease Arrest (Cat# 786-108F):** A protease cocktail specifically developed for sample preparation for 2D-studies.

NOTE: For other related products, visit our web site at www.GBiosciences.com or contact us.