# ProteSEEKER™

**G-Biosciences**

## Abstract

This protocol is for the ProteSEEKER™ kit, designed to identify the specific type of proteases present in cell or tissue lysates, and allows determining the minimal number of individual protease inhibitors required for a sample.

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## Guidelines

### 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 twelve 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 12 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)

| Description | Size |
|---|---|
| Protease Substrate (Lyophilized; 150µl) | 1 |
| Incubation Buffer | 5ml |
| Precipitation Agent | 5ml |
| Assay Buffer | 6ml |

***Protease Inhibitor Set***

| Inhibitor | Inhibits | Size |
|---|---|---|
| **AEBSF [100X]** 4-(2-Aminoethyl)- benzenesulfonyl-fluoride, hydrochloride | Irreversible inhibitor of serine proteases- trypsin, chymotrypsin, plasmin, plasma kallikrein, & thrombin | 25µl |
| **ALLN [100X]** (N-Acetyl-Leu-Leu-Nle-CHO (Calpain Inhibitor I)) | Cell-permeable, peptide aldehyde inhibitor of Calapin and other neutral cysteine proteases | 25µl |
| **Antipain-dihydrochloride [100X]** | Inhibits papain, trypsin and to small extent plasmin | 25µl |
| **Aprotinin [100X]** | Inhibits serine proteases- plasmin, kallikrein, trypsin, chymotrypsin | 25µl |
| **Bestatin [100X]** | Inhibitor of amino-peptidases and other exopeptidases (does not inhibit carboxypeptidases) | 25µl |
| **Chymostatin [100X]** | Specific inhibitor of α, β, γ, δ chymotrypsin | 25µl |
| **E-64 [100X]** (N-(N-(L-3 Trans-carboxirane- 2-carbonyl)-L-leucyl)- agmatine) | Inhibitor of papain and other cystein proteases like cathepsin B & L | 25µl |
| **EDTA-$Na_2$ [100X]** | Inhibits metalloproteases | 25µl |
| **Leupeptin [100X]** | Inhibits Serine & Cystein proteases e.g. Plasmin, Trypsin, Papain & Cathepsin B | 25µl |
| **Pepstatin [100X]** | Inhibits Aspartic proteases, such as pepsin, renin, cathepsin D, chymosin | 25µl |
| **Phosphoramidon [100X]** | Specifically inhibits thermolysin,collagenase & other metalloendoproteinases | 25µl |
| **PMSF [100X]** Phenylmethylsulfonyl fluoride | Inhibits serine proteases; chymotrypsin, trypsin & thrombin, also inhibits cystein proteases such as papain (reversible by DTT) | 25µl |

## 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.

## ADDITIONAL ITEMS REQUIRED

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.

**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:

$$\% \text{ Protease Activity Present} = \frac{\text{OD Test}}{\text{OD Control}} \times 100$$

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

Use the result and the Protease Inhibitor Set table above to determine the protease species present in your sample. Individual protease inhibitors are available in larger quantities for preparative scale use.

## Materials

ProteSEEKER™ 786-325 by G-Biosciences

## Protocol

PREPARATION BEFORE USE

**Step 1.**

***Protease Substrate:*** Reconstitute the supplied Protease Substrate by adding 150µl water into the vial, mix it to dissolve completely.

PREPARATION BEFORE USE

**Step 2.**

After reconstitution, store the substrate at -20°C.

PREPARATION BEFORE USE

**Step 3.**

***Inhibitors:*** Allow the solutions to warm to room temperature, vortex the vial for 15-20 seconds and then briefly centrifuge before use.

DURATION
00:00:15

PREPARATION BEFORE USE

**Step 4.**

Add appropriate volume of 100X inhibitor solution(s) in the samples to be tested to give a final 1X concentration.

**Step 5.**

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

| 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 |

NOTES
**Colin Heath** 15 Jun 2016

***NOTE:*** All twelve 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.

**Step 6.**

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

DURATION
02:00:00

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

**Step 7.**

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

DURATION
00:10:00

**Step 8.**

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

**Step 9.**

Transfer the supernatant to clean tubes.

**Step 10.**

Add 120µl Assay Buffer to each tube and mix.

NOTES
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A pink color will develop instantly. The intensity of the color developed in each tube is proportional to protease activity.

***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.

**Step 11.**

Read the reaction color at 574nm against the blank.

NOTES
**Colin Heath** 12 Sep 2016

See the Guidelines for calculation of percent inhibition.