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A Geno Technology, Inc. (USA) brand name

Protease Assay™

(Cat. # 786-028)

INTRODUCTION 3
ITEM(S) SUPPLIED 3
STORAGE CONDITIONS 3
ITEMS NEEDED BUT NOT SUPPLIED 3
PREPARATION BEFORE USE 4
SUBSTRATE 4
PROTOCOL 4
PREPARATION OF TRYPSIN STANDARD CURVE: 4
PROTEASE ASSAY- STANDARD CURVE: 5
RELATED PRODUCTS 5

INTRODUCTION

All live cells and tissues contain proteases, which are liberated during the processing of samples. Sensitive protease assays are needed to study protease activity present in the sample of interest. G-Biosciences Protease Assay Kit is designed for the quantitative determination of proteases present in the protein sample, and uses a dye- labeled protein substrate. The proteases present in the sample of interest will digest the protein substrate and release dye labeled peptides. The absorbance of the dye-labeled peptide is measured at 570nm for determination of protease activity. Chemically stabilized Trypsin is supplied with the kit as a general protease standard; however, other specific protease standard can also be used.

G-Biosciences Trypsin, Mass Spectrometry Grade is an ultra-pure trypsin from bovine pancreas, modified by methylation followed by TPCK treatment and is extremely resistant to autolysis. The kit components are sufficient for 50 assays in a microtiter plate format or 0.5ml assay tubes.

ITEM(S) SUPPLIED (Cat. # 786-028)

Description	Size
Incubation Buffer	5ml
Precipitation Agent	5ml
Assay Buffer	6ml
Protease Substrate (lyophilized)	1 vial (150µl)
Trypsin, Mass Spectrometry Grade (bovine)	3 x 20µg each
Trypsin Suspension Buffer	2 x 0.5ml

STORAGE CONDITIONS

The kit is shipped on blue ice. Upon arrival, store all components of the kit at 4°C, except Trypsin, Mass Spectrometry Grade, which should be stored at -20° C. When stored and used properly the kit is stable for 1 year.

ITEMS NEEDED BUT NOT SUPPLIED

- 96 well titer plates
- Micro-centrifuge tubes
- Micro-centrifuge
- Micro-plate reader or Spectrophotometer.

PREPARATION BEFORE USE

Substrate

Reconstitute the supplied Protease Substrate (dye-labeled protein) by adding 150µl DI water into the vial, mix it to dissolve completely. After reconstitution, store the substrate at -20°C and is stable for up to 6 months.

> ***NOTE:*** *The assay is designed for 96 well titer plate. The use of 96 well titer plate requires use of a centrifuge adapted for 96 well titer plate. Alternatively, the assay may be performed in micro-centrifuge tubes and the final reaction product is transferred to a 96 well titer plates for the measurement of optical density.*

PROTOCOL

Preparation of Trypsin Standard curve:

1. Dissolve the supplied Trypsin (20µg) in 250µl Trypsin Suspension Buffer (stock trypsin, 80ng/µl) and serially dilute it with Incubation Buffer to get dilutions from 20ng/µl to 1.25ng/µl
   NOTE: *Any unused stock trypsin may be stored at –70º C and can be used within two weeks. Avoid repeated freeze-thaw cycle after trypsin reconstitution.*
2. Prepare the reaction mix for unknown sample for protease assay and trypsin standard in duplicate as follows:

	Blank	**Trypsin standard 1.25ng/µl to 20ng/µl**	**Unknown Sample**
Unknown Sample (select an appropriate volume between 1-45µl)	-	10µl	1-45µl
Protease Substrate Solution	2.5µl	2.5µl	2.5µl
Add Incubation Buffer or a buffer of your choice to adjust the final assay volume to 50µl (Final volume of the reaction mixture is 50µl)	- to 50µl	to 50µl	-to 50µl

3. Seal the plate or tube and incubate at 37°C for 2 ½ hours
   NOTE: *For slow acting proteases, incubation time may be extended for up to 24 hours.*
4. After incubation, add 50µl Precipitation Agent, mix the contents and incubate again at 37°C for 10 minutes.
5. Centrifuge the tubes at 12,000xg for 5 minutes (for 96 well titer plate 2-4,000xg for 15 minutes).
6. Transfer 80µl supernatant to clean tubes or wells without disturbing the pellet. Add 120µl Assay Buffer in each tube/well and mix. A pink color will develop instantly. Read the reaction color at 570nm against the blank. The intensity of the color

developed in each tube is proportional to the protease activity, which may be assessed using the standard calibration curve.

NOTE: *If the assay is performed in microfuge tubes, transfer the reaction product to a 96 well titer plate and read the color at 570nm against the blank. Alternatively, use a micro-cuvette to read the color. The reaction product may be diluted with water to 1ml and read in 1ml cuvette; however, the sensitivity will drop by 4-5 fold. Alternatively, the reaction volume of each component may be increased to 4-5 folds to give 1ml total reaction volume.*

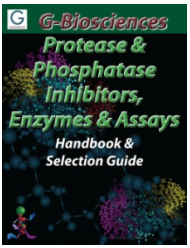
PROTEASE ASSAY- STANDARD CURVE:

When quantitating the protease activity, a protease activity standard curve may be generated with the supplied Trypsin, Mass Spectrometry Grade.

NOTE: *TPCK trypsin generally serve as a standard for relative comparison of overall protease activity in different samples. However, for specific protease activity, preparation of standard curve with specific protease of interest is recommended.*

RELATED PRODUCTS

Download our Protease & Phosphatase Inhibitors, Enzyme & Assays Handbook.



http://info.gbiosciences.com/protease-phosphatase-inhibitors-enzymes-assay-handbook

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