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A Geno Technology, Inc. (USA) brand name

# Fluoro™ Protease Assay

(Cat. # 786-320)



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## INTRODUCTION

G-Biosciences Fluoro™ Protease Assay Kit is designed for the quantitative determination of proteases present in the protein sample. The assay uses fluorescein isothiocyanate (FITC)-labeled casein as a general protease substrate. The fluorescein label on the FITC-Casein is highly quenched. When the proteases present in the sample of interest digest the FITC-Casein substrate into smaller peptides, the quenching of the fluorescence label is removed and the fluorescence of the substrate is increased. The fluorescence of the FITC-labeled peptide is measured with excitation at 485nm and emission at 530nm to determine protease activity. The kit detects nanogram level of proteases present in the sample.

The kit is supplied with our chemically stabilized Trypsin, Mass Spectrometry Grade for use as a general protease control. However, other specific protease standard control can also be used. Trypsin, Mass Spectrometry Grade is an ultra-pure trypsin from porcine pancreas, modified by methylation followed by TPCK treatment and is extremely resistant to autolysis. The kit components are sufficient for 1,000 assays in a microtiter plate format.

## ITEM(S) SUPPLIED (Cat. # 786-320)

Description	Size
Fluoro™ Assay Buffer [10X]	30ml
FITC-Casein Protease Substrate	1mg vial
Trypsin, Mass Spectrometry Grade (Porcine)	3 x 20µg vial
Trypsin Suspension Buffer	2 x 0.5ml

## STORAGE CONDITION

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

## ADDITIONAL ITEMS REQUIRED

1. Fluorometer
2. 96 well Fluorometer compatible titer plates

## PREPARATION BEFORE USE

### **Fluoro™ Assay Buffer**

1. Dilute the Fluoro™ Assay Buffer 1:10 with deionized water to create a 1X working solution of Fluoro™ Assay Buffer. For every 10ml buffer, add 1ml Fluoro™ Assay Buffer to 9ml deionized water.
2. Store the 1X Fluoro™ Assay Buffer at 4°C. The reconstituted substrate is stable for up to 12 months.

### **FITC-Casein Protease Substrate**

1. Reconstitute the supplied FITC-Casein Protease Substrate by adding 200µl 1X Fluoro™ Assay Buffer to the vial and mixing to dissolve completely (stock substrate, 5µg/µl).
2. Store the reconstituted substrate at -20°C. The reconstituted substrate is stable for up to 6 months.

**NOTE:** *If the FITC-Casein Substrate is subjected to repeat freeze-thaw cycles a slight increase in background will occur. Aliquot the FITC-Casein Substrate and store at -20°C protected from light.*

3. Immediately before performing the assay, dilute the 5µg/µl stock substrate solution 500 fold to a final concentration of 0.01µg/µl with 1X Fluoro™ Assay Buffer. Each assay requires 1µg FITC-Casein Protease Substrate.
4. NOTE: The assay is designed for 96 well titer plates. Alternatively, the assay may be performed in micro-centrifuge tubes and the final reaction product is transferred to a 96 well titer plates or diluted and read with standard cuvettes for the measurement of fluorescence.

## PROTOCOL

### Preparation of Trypsin Standard curve

1. Add 200µl Trypsin Suspension Buffer to a vial of 20µg Trypsin and incubate at room temperature for 15 minutes with periodic vortexing. Once fully dissolved a 100ng/µl stock solution is achieved. Make serial dilutions in the range of 0.5ng/µl to 0.01ng/µl with 1X Fluoro™ Assay Buffer as indicated in the table below. Each assay requires 100µl serially diluted Trypsin standard. We recommend performing duplicates of each standard

**NOTE:** Any unused stock trypsin may be stored at -70°C and can be used within four weeks. Avoid repeated freeze-thaw cycle after trypsin reconstitution.

Tube #	Trypsin Standard (ng/µl)	Trypsin	1X Fluoro™ Assay Buffer (µl)
A	0.5	10µl 100ng/µl stock solution	1990
B	0.4	400µl Tube A	100
C	0.3	300µl Tube A	200
D	0.2	200µl Tube A	300
E	0.1	100µl Tube A	400
F	0.05	50µl Tube A	450
G	0.01	10µl Tube A	490

2. Prepare samples in the 96-well plate wells as indicated below.

	FITC-Casein Substrate (µl)	1X Fluoro™ Assay Buffer (µl)	Standard/ Unknown Sample (µl)
Blank	100	100	---
Trypsin Standards	100	---	100
Unknown sample	100	To 200µl	1-10

3. Seal the plate, mix and incubate 30 minutes to 2 hours at room temperature.  
**NOTE:** Avoid vigorously mixing the samples as excessive turbulence can lead to high fluorescent backgrounds.
4. Measure the fluorescence at 485nm excitation and 530nm emission.  
**NOTE:** If the assay was performed in microfuge tubes either transfer 200µl samples to a 96-well plate and read or add 0.8ml 0.5M Tris buffer, pH8.5 to the samples in tubes and read in fluorescent compatible cuvettes.

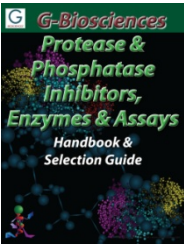
## PROTEASE ASSAY STANDARD CONTROL

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

NOTE: Trypsin, Mass Spectrometry Grade generally serves as a standard for relative comparison of overall protease activity in different test samples. 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>

For other related products, visit our website at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.

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