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Fluoro™ Protease Assay

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 relieved and the fluorescence of the substrate is increased. The fluorescence of the FITC-labeled peptide is measured with excitation at 485nm and emission at 535nm to determine protease activity. The kit detects picogram level of proteases present in the sample.

The kit is supplied with our chemically stabilized MSG Trypsin-Porcine for use as a general protease control. However, other specific protease standard control can also be used. MSG-trypsin 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.

KIT COMPONENTS Cat # 786-320

Fluoro™ Assay Buffer	50ml
FITC- Casein Protease Substrate	1mg vial
MSG Trypsin – Porcine	3 x 20µg vial
Trypsin Suspension Buffer	2 x 0.5ml

STORAGE CONDITION

Shipped at ambient temperature. Store all components of the kit at 4°C upon receiving, except MSG Trypsin-Porcine, 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 fluorometer compatible titer plates, micro-centrifuge tubes, fluorometer

PROTOCOL

Preparation Before Use:

FITC- Casein Protease Substrate -

Reconstitute the supplied FITC-Casein Protease Substrate by adding 200µl Fluoro™ Assay Buffer into the vial and mixing it to dissolve completely (stock substrate, 5µg/µl). After reconstitution, store the substrate at -20°C and is stable for up to 6 months. Dilute the stock substrate solution 500 fold to 0.01µg/µl with Fluoro™ Assay Buffer as needed before set up the assays. Each assay requires 1µg FITC- Casein Protease Substrate.

Note: The assay is designed for 96 well titer plate. The use of 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 fluorescence.

Preparation of Trypsin Standard curve:

Dissolve the supplied Trypsin (20µg) in 200µl Trypsin Suspension Buffer (stock trypsin, 100ng/µl). Make serial dilutions in the range of 0.5ng/µl to 0.01ng/µl with Fluoro™ Assay Buffer as needed before set up the assays. Each assay requires 100µl serially diluted Trypsin 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.



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Prepare the reaction mix for unknown sample for protease assay and Trypsin standard in duplicate as follows:

	Blank	Trypsin standard 0.01ng/μl to 0.5ng/μl	Unknown Sample
Unknown Sample (select an appropriate volume between 1-10μl)	-----	100μl	1-10μl
FITC-Casein Substrate Solution (0.01μg/μl)	100μl	100μl	100μl
Add Fluoro™ Assay Buffer to adjust the final assay volume to 200μl	100μl	-----	to 200μl

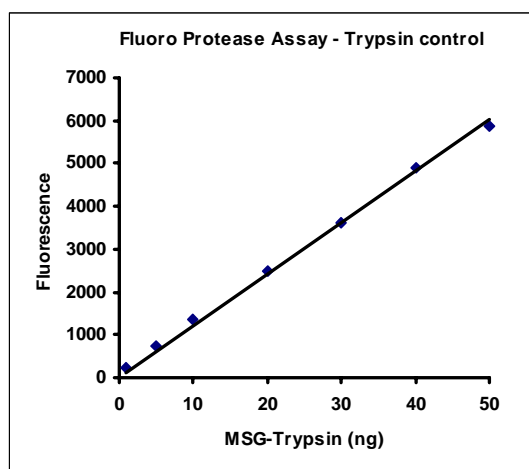
Seal the plate or tube and incubate 30minutes to 2 hours at room temperature.
Remove the cover of the plate or transfer the samples from tubes to the plate wells.
Measure the fluorescence at 485nm excitation and 530nm emission.

Protease Assay Standard Control:

When quantitating the protease activity, a protease activity standard curve may be generated with the supplied MSG-Trypsin.

Note: MSG-Trypsin generally serve as a standard for relative comparison of overall protease activity in different tsamples. However, for specific protease activity, preparation of standard curve with specific protease of interest is recommended.

Note: For Protease Inhibitor Cocktail and Individual Protease Inhibitors, please see Related Products.



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 etc.
- 2. Focus Protease Arrest (Cat # 786-108F):** A protease inhibitor cocktail specifically prepared for IEF/2D sample preparation.
- 3. ProteCEASE 50 and 100 (Cat# 786-326, 786-327, 786-328, 786-334, 786-335, and 786-336):** Protease inhibitor cocktail for large volumes of extraction/ lysis buffers and is supplied 10 vials /kit or 20 vials/kit, each vial suitable for 50ml or 100ml extraction /lysis buffer.

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

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