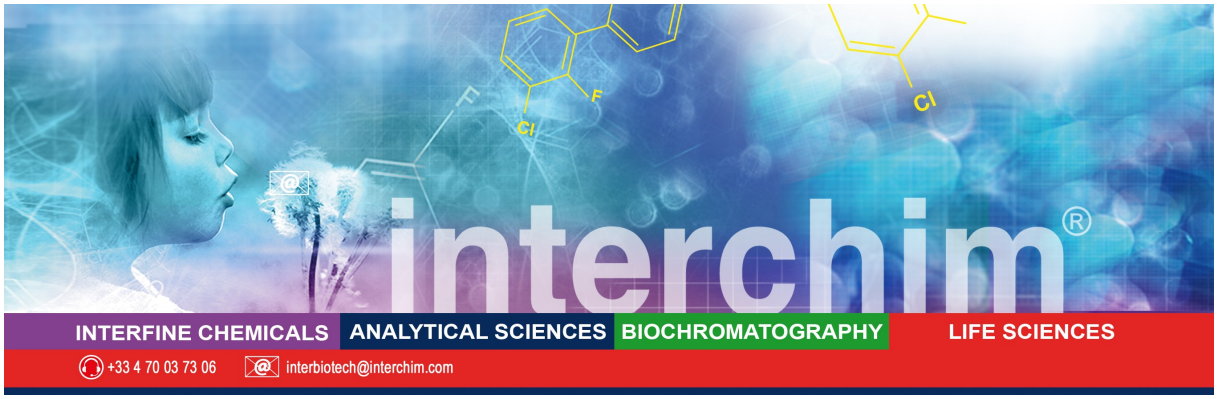
FT-BE928A



Casein

Protease assay substrate

Product Information

Name :	Casein-TAMRA
Catalog Number :	FP-BE928A, 5 mg
Solubility:	Dionized water, aquous buffer or 1 M NaOH
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 546/574 nm

Name :	Casein-Fluorescein
Catalog Number :	FP-65802B, 5 mg
Solubility:	Dionized water, aquous buffer or 1 M NaOH
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 494/521 nm

Storage: –20°C >1 year

Introduction

Casein is a phosphoprotein found in milk. This product contains α-, β-, γ-, and κ-casein subunits. In the intact substrate, casein is heavily labeled with TAMRA, resulting in almost total quenching of the conjugate's fluorescence. Compared to the fluoresceinated casein, TAMRA-labeled casein demonstrates pH-insensitive fluorescence. It is readily used for continuous fluorometric measurement of protease activity. Protease-catalyzed hydrolysis relieves this quenching conjugate, yielding brightly green fluorescent dye-labeled peptides. The increase in fluorescence intensity is directly proportional to protease activity. The lightly TAMRA-labeled casein may be useful for a continuous assay if monitored by fluorescence polarization.

Directions for use

For a proteolytic assay[1], incubate the fluorescent conjugated casein with proteases in appropriate assay buffer. The protease activity is demonstrated by the increment of fluorescence. A detail protocol can be found in Universal Fluorimetric Protease Assay Kit (Cat# BK962A).

Sample Protocol For Trypsin

1. Make a 5-10mg/mL Casein, fluorescent conjugated stock solution in PBS buffer. Unused stock solution can be divided into single use aliquots and stored at -20°C, and avoid exposure to light.
2. Prepare 2X assay working solution by diluting the fluorescent conjugated stock solution into 50-100 mM Tris buffer (pH 7.4) at 100-400 µg/mL

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Note1: The 2X Assay working solution is designed for detecting the activity of chymotrypsin, trypsin, thermolysin, proteinase K, protease XIV, and human leukocyte elastase. For other proteases, please refer to Appendix I for the appropriate assay buffer formula.

Note2: The optimum concentration of the assay working solution should be determined experimentally for individual proteases.

3. Mix equal volume of the trypsin standards or samples with 2X Assay working solution.
4. Monitor the fluorescence increase at Ex/Em = 490/525 nm for Fluorescein or 546/574 nm for TAMRA.
   For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30 minutes.
   For end-point reading: Incubate the reaction at a desired temperature for 30 to 60 minutes,protected from light. Then measure the fluorescence intensity.

Appendix I

Protease	*1X Assay Buffer**
Cathepsin D	20 mM Sodium Citrate, pH 3.0
Papain	20 mM sodium acetate, 20 mM cysteine, 2 mM EDTA, pH 6.5
PAE	20 mM sodium phosphate, pH 8.0
Pepsin	10 mM HCl, pH 2.0
Porcine pancreas elastase	10 mM Tris-HCl, pH 8.8
Subtilisin	20 mM potassium phosphate buffer, pH 7.6, 150 mM NaCl

References

1. **Twining SS** (1984). Fluorescein isothiocyanate-labeled casein assay for proteolytic enzymes. Anal Biochem 143, 30-4.
2. **Arthur JS and Mykles DL** (2000). Calpain zymography with casein or fluorescein isothiocyanate casein. Methods Mol Biol. 144, 109-16
3. **Farmer WH and Yuan ZY** (1991). A continuous fluorescent assay for measuring protease activity using natural protein substrate. Anal Biochem 197, 347-52

Related Product

- Universal Fluorimetric Protease Assay Kit (Green), BK962A
- Universal Fluorimetric Protease Assay Kit (Red), BK963A

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06