Casein, FITC-conjugated

Catalog number: 13440
Unit size: 5 mg

Component	Storage	Amount
Casein, FITC-conjugated	Freeze (<-15 °C), Minimize light exposure	5 mg

OVERVIEW

Casein is considered to be a generic substrate for a broad spectrum of proteases. As native casein this fluoresceinated casein is hydrolyzed by many proteases, and widely used for fluorimetric measurement of protease activity. In the intact substrate, casein is heavily labeled with 5-FITC, resulting in significant fluorescence quenching. Protease-catalyzed hydrolysis relieves its quenching effect, yielding brightly green fluorescent dye-labeled short peptides. The increase in fluorescence intensity is directly proportional to protease activity. We do not recommend that this conjugate be used for fluorescence polarization assay. For fluorescence polarization we can custom-make the lightly labeled fluorescein casein conjugate.

AT A GLANCE

Chemical Properties of Casein
Appearance: Light yellow powder
Excitation/Emission: 494/521 nm
Solvent: Water

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Casein, FITC-conjugated stock solution:
Make a 5 - 10 mg/mL Casein, FITC-conjugated stock solution in PBS buffer.

PREPARATION OF WORKING SOLUTION

Casein FITC-conjugated working solution (2X):
Dilute the FITC-conjugated stock solution into 50 - 100 mM Tris buffer (pH 7.4) at 100 - 400 µg/mL. 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 Table 1 for the appropriate assay buffer formula. The optimum concentration of the assay working solution should be determined experimentally for individual proteases.

SAMPLE EXPERIMENTAL PROTOCOL

1. Mix equal volume of the trypsin standards or samples with 2X Assay working solution.

2. Monitor the fluorescence increase at Ex/Em = 490/525 nm.

   a. For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30 minutes.

   b. For end-point reading: Incubate the reaction at a desired temperature for 30 to 60 minutes, protected from light. Then measure the fluorescence intensity.

Table 1. Appropriate assay buffer formula for Assay working solution.

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

EXAMPLE DATA ANALYSIS AND FIGURES

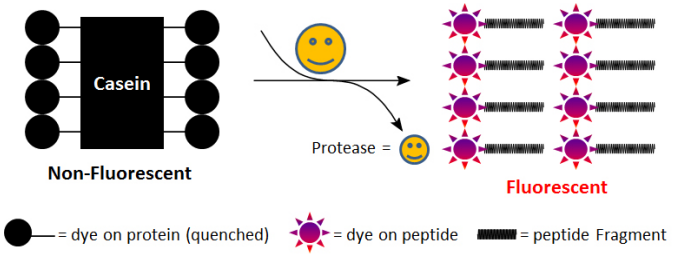

Figure 1.

Proteases hydrolyze the quenching effect of the labeled 5-FITC, resulting in a bright green fluorescence proportional to protease activity.

DISCLAIMER