

## Casein, TAMRA-conjugated

 Catalog number: 13441  
 Unit size: 5 mg

Component	Storage	Amount
Casein, TAMRA-conjugated	Freeze (< -15 °C), Minimize light exposure	1 vial (5 mg)

### OVERVIEW

Casein is considered to be a generic substrate for a broad spectrum of proteases. As native casein this rhodaminated casein is hydrolyzed by many proteases, and widely used for fluorometric measurement of protease activity. In the intact substrate, casein is heavily labeled with TAMRA, resulting in significant fluorescence quenching. Protease-catalyzed hydrolysis relieves its quenching effect, yielding brightly orange fluorescent dye-labeled short peptides. The increase in fluorescence intensity is directly proportional to protease activity. Compared to FITC-labeled casein, this casein substrate has pH-independent fluorescence. This feature is more convenient for the assays that require low pH. 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.

### 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, TAMRA-conjugated stock solution

Make a 2.5 - 5 mg/mL Casein, TAMRA-conjugated stock solution in PBS buffer.

**Note** Unused stock solution can be divided into single use aliquots and stored at -20 °C, and avoid exposure to light.

### PREPARATION OF WORKING SOLUTION

#### Casein TAMRA-conjugated working solution (2X)

Dilute the TAMRA-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.

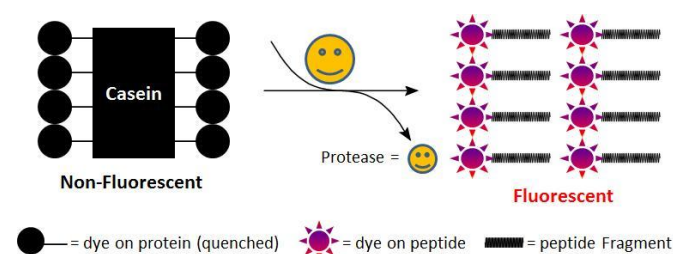
### SAMPLE EXPERIMENTAL PROTOCOL

**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

- Mix equal volume of the trypsin standards or samples with 2X Assay working solution.
- Monitor the fluorescence increase at Ex/Em = 540/590 nm.
  - 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.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.**

Proteases hydrolyze the quenching effect of the labeled TAMRA, resulting in a bright yellow-orange fluorescence proportional to protease activity.

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