

Universal Protease Substrate

Casein, resorufin-labeled

Cat. No. 1 080 733 15 mg

Cat. No. 1 734 334 40 mg

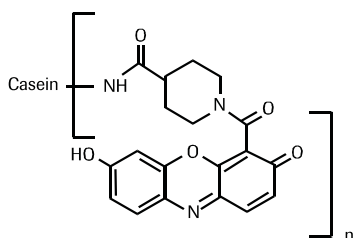
Version 3, August 1999

Store at -15 to -25° C

Product description

Preparation Casein from cow milk was coupled with activated resorufin [N-(resorufin-4-carbonyl)piperidine-4-carboxylic acid N-hydroxysuccinimide ester*] and purified by gel chromatography. Approx. 90 µg resorufin are bound to 1 mg casein (control by total hydrolysis using pronase*).

Structure



Stability

Stable at -15 to -25° C, stored dry and protected from light. An aqueous solution is stable for several months at -15 to -25° C and for 2-3 days at 2-8° C. It is recommended to store aqueous solutions in aliquots at -15 to -25° C.

Spectral properties of the hydrolyzed substrate

Absorption (excitation) maximum in the neutral and alkaline range $\lambda = 574 \text{ nm}$, $\epsilon = 66000 \text{ [l} \times \text{mol}^{-1} \times \text{cm}^{-1}]$, in the acidic range $\lambda = 467 \text{ nm}$. Emission maximum in the neutral and alkaline range $\lambda = 584 \text{ nm}$, in the acidic range $\lambda = 559 \text{ nm}$.

Application

The preparation is a general substrate for proteases and is especially well suited for the detection of traces of protease activities. It can be used in a homogeneous assay and can be measured both spectrophotometrically and fluorimetrically.

Principle

By treatment with proteases, resorufin-labeled peptides are released from casein, resorufin-labeled. They cannot be precipitated by trichloroacetic acid. The concentration of these resorufin-labeled peptides in the supernatant is equivalent to the proteolytic activity present.

Application example for the determination of proteolytic activity modified according to Twining (1).

Solutions/reagents

- I. Substrate solution
0.4% Casein, resorufin-labeled (w/v) in redist. water.
- II. Incubation buffer
0.2 M Tris-HCl pH 7.8, 0.02 M CaCl₂.
- III. Sample solution
- IV. Stop reagent
5% Trichloroacetic acid (w/v) in redist. water.
- V. Assay buffer
0.5 M Tris-HCl, pH 8.8.

Assay procedure

Wavelength: 574 nm (absorbance);
584 nm (emission)
Light path: 1 cm
Incubation temperature: 37° C

Pipette into reaction vessels (1 ml)	sample blank	sample
substrate solution (I)	50 µl	50 µl
incubation buffer (II)	50 µl	50 µl
redist. water	100 µl	-
sample solution (III)	-	100 µl
Incubate at 37° C, for a suitable space of time (15 min till overnight). Stop reaction by addition of		
stop reagent (IV)	480 µl	480 µl
Incubate for 10 min at 37° C, subsequently centrifuge for 5 min and pipette into Sarstedt cuvettes		
supernatant	400 µl	400 µl
assay buffer (V)	600 µl	600 µl
mix and immediately read absorbance of the sample against blank at 15-25° C (= ΔA sample).		

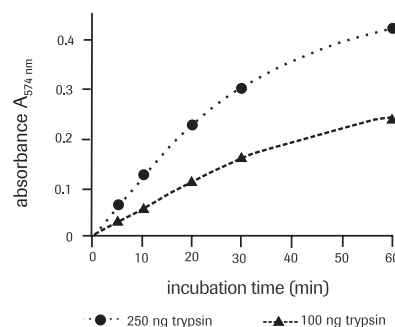


Fig. 1: Influence of the incubation time on the Casein-resorufin hydrolysis by trypsin

Results with different proteases

Limited and complete (exhaustive) digestion of casein-resorufin by different proteases

- a) Digestion by small amounts of proteases for 15 min (determination of the detection limit)**
- b) Digestion by large amounts or proteases overnight (maximum of total hydrolysis)**

Enzyme	enzyme-amount	$\Delta_0 D_{574 \text{ nm}}$	enzyme-amount	absorbance $\Delta E_{574 \text{ nm}}$
pronase*	0.1 μg	0.11	1 mg	1.9
trypsin, sequencing grade*	0.1 μg	0.07	20 μg	1.06
endoproteinase Asp-N, sequencing grade*	0.1 μg	0.09	10 μg	1.3
endoproteinase Lys-C, sequencing grade*	-	-	5 μg	0.39

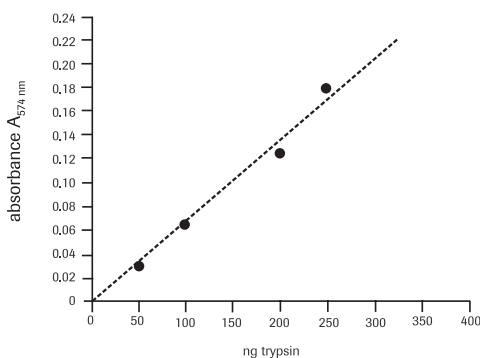


Fig. 2: Hydrolysis of casein-resorufin by different amounts of trypsin

Simplified procedure for carrying out protease tests with resorufin-labeled casein to detect high protease concentrations in solutions

Procedure (carried out in a 1.5 ml plastic Eppendorf tube)

- Pipette into the tube:
- 0.05 ml resorufin-labeled casein (4 mg/ml H₂O)
 - 0.05 ml 0.2 M Tris-HCl buffer, pH 7.8, 0.02 M calcium chloride
 - 0.1 ml protease solution

Mix and follow the color change at 15-25°C in comparison with a blank which contains water instead of the protease solution. The color changes within a short time from bluish-violet to red, if sufficient protease activity is present. The observation of the color change is best carried out vertically through the open Eppendorf tube against a white sheet of paper.

Some results obtained with the use of various concentrations of protease:

Concentration	Color change within
2 mg/ml	about 1 min
0.5 mg/ml	about 5 min
0.2 mg/ml	about 10 min

References

- Twining, S. S. (1984) *Anal. Biochem.* **143**, 30-34.
- Schickaneder, E. et al (1988) "Casein-resorufin, a new substrate for a highly sensitive protease assay" *Fresenius Z. Anal. Chem.* **330**, 360.

* available from Roche Molecular Biochemicals

** The detection limit can be lowered by using fluorimetric analysis or by increasing the incubation time (e.g. overnight).

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