

Universal Protease Substrate

Casein, resorufin-labeled

Cat. No. 11 080 733 001

Cat. No. 11 734 334 001

15 mg

40 mg

Version February 2006

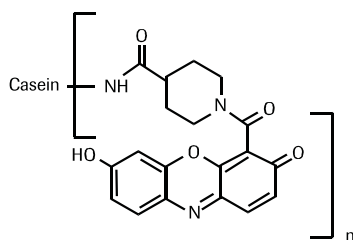
Store at -15 to -25°C

1. What this Product Does

Contents

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



Storage and 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 $+2$ to $+8^{\circ}\text{C}$. 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 $\lambda = 574$ nm, $\epsilon = 66000$ [$\text{l} \times \text{mol}^{-1} \times \text{cm}^{-1}$], in the acidic range $\lambda = 467$ nm. Emission maximum in the neutral and alkaline range $\lambda = 584$ nm, in the acidic range $\lambda = 559$ 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.

2. How to Use this Product

2.1 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 double dist. water.

II. Incubation Buffer

- 0.2 M Tris-HCl pH 7.8, 0.02 M CaCl_2 .

III. Sample Solution

IV. Stop Reagent

- 5% Trichloroacetic acid (w/v) in double dist. 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: $+37^{\circ}\text{C}$

Pipette into reaction vessels (1 ml)	sample blank	sample
Substrate Solution(I)	50 μl	50 μl
Incubation Buffer(II)	50 μl	50 μl
double dist. water	100 μl	–
Sample Solution(III)	–	100 μl

Incubate at $+37^{\circ}\text{C}$, for a suitable space of time (15 min till overnight). Stop reaction by addition of

Stop Reagent (IV)	480 μl	480 μl
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Incubate for 10 min at $+37^{\circ}\text{C}$, 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 $+15$ to $+25^{\circ}\text{C}$ ($= \Delta A$ sample).

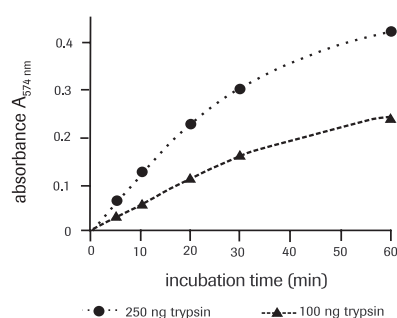


Fig. 1: Influence of the incubation time on the Casein-resorufin hydrolysis by trypsin

2.2 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 to +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

3. 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 of proteases overnight (maximum of total hydrolysis)**

Enzyme	enzyme-amount	ΔOD_{574} nm	enzyme-amount	absorbance ΔA_{574} nm
Pronase*	0.1 μ g	0.11	1 mg	1.9
Trypsin, Sequencing Grade*	0.1 μ g	0.07	20 μ g	1.06
Endoproteinase Asp-N, Sequencing Grade*	0.1 μ g	0.09	10 μ g	1.3
Endoproteinase Lys-C, Sequencing Grade*	-	-	5 μ g	0.39

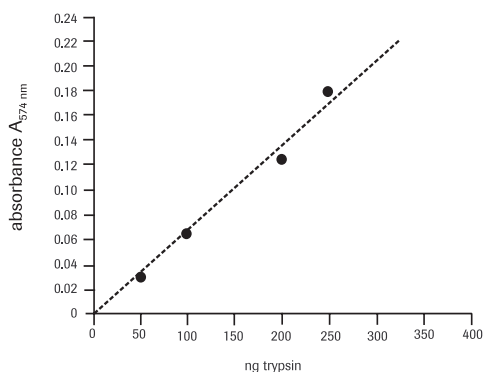


Fig. 2: Hydrolysis of casein-resorufin by different amounts of trypsin

4. References

- 1 Twining, S. S. (1984) *Anal. Biochem.* **143**, 30–34.
- 2 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 Applied Science

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

Patent 0209875 and US 4.954.630 owned by Roche Diagnostics GmbH.

5. Supplementary Information

5.1 Ordering Information

Product	Pack size	Cat. No.
Pronase	1 g	10 165 921 001
	5 g	11 459 643 001
Trypsin Sequencing Grade	4 x 25 μ g	11 418 475 001
	4 x 100 μ g	11 047 841 001
Endoproteinase Asp-N Sequencing Grade	2 μ g	11 420 488 001
	3 x 2 g	11 054 389 001
Endoproteinase Lys-C Sequencing Grade	5 μ g	11 420 429 001
	3 x 5 μ g	11 047 825 001

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