For life science research only.
Not for use in diagnostic procedures.

Universal Protease Substrate
Casein, resorufin-labeled

Version: 21
Content Version: June 2021

Cat. No. 11 734 334 001 40 mg

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Universal Protease Substrate, Casein, resorufin-labeled	▪ Casein from cow's milk was coupled with activated resorufin [N-(resorufin-4-carbonyl)piperidine-4-carbonic acid N-hydroxysuccinimide ester] and purified by gel chromatography. ▪ Approximately 90 µg resorufin are bound to 1 mg casein (control by total hydrolysis using Pronase*).	1 vial/ 40 mg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Universal Protease Substrate	Store at −15 to −25°C. ⚠ ***Store dry.*** ⚠ ***Keep protected from light.***

Storage Conditions (Working Solution)

Stability of aqueous solutions

Temperature [°C]	Stability
−15 to −25	For several months.
+2 to +8	2 to 3 days
+15 to +25	Product is rapidly hydrolyzed in solution.

⚠ ***Store aqueous solutions in aliquots at −15 to −25°C to avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For preparation of lyophilizate

- Double-distilled water

For the proteolytic activity

- Microtubes, 1 ml
- Tris-HCl*
- $CaCl_2$
- Trichloroacetic acid 5% (w/v)

For the protease concentration

- Microtubes, 1.5 ml
- Tris-HCl*
- $CaCl_2$
- Protease solution

1.4. Application

Use Universal Protease Substrate for the detection of traces of protease activities.
It can be used in a homogeneous assay and can be measured spectrophotometrically and fluorometrically.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Solution	Composition/Preparation	For use in...
Substrate solution	0.4% Casein, resorufin-labeled (w/v) in double-distilled water.	Reaction mix
Incubation buffer	0.2 M Tris-HCl*, pH 7.8, 0.02 M $CaCl_2$.	Reaction mix
Stop reagent	5% Trichloroacetic acid (w/v) in double-distilled water.	Stopping the reaction.
Assay buffer	0.5 M Tris-HCl*, pH 8.8.	Mix for absorbance reading.

2.2. Protocols

Application example for the determination of proteolytic activity

Apply the following photospectrometer parameters:

Wave length	574nm (absorbance) 584 nm (emission)
Light path	1 cm
Temperature	+37°C

1. Pipette into 1 ml reaction vessels:

Solution	Sample [µl]	Blank [µl]
Substrate solution	50	50
Incubation buffer	50	50
Double-distilled water	–	100
Sample solution	100	–

2. Incubate at +37°C for 15 minutes to overnight.

3. Add 480 µl Stop reagent to Sample and Blank to stop the reaction.

4. Incubate at +37°C for 10 minutes.
   – Centrifuge for 5 minutes.

5. Pipette 400 µl of supernatant into a cuvette.

6. Add 600 µl of Assay buffer.
   – Mix and immediately read absorbance of the sample against Blank at +15 to +25°C (= ΔA sample).

Detection of high protease concentrations in solutions

Below is a simplified protocol for carrying out protease tests with resorufin-labeled casein.

1. Pipette into 1.5 ml microtubes:

Solution	Sample [µl]	Blank [µl]
Substrate solution	50	50
Incubation buffer	50	50
Protease solution	100	–
Double-distilled water	–	100

2. Mix and follow the color change at +15 to +25°C in comparison to Blank.
   The color changes within a short time from bluish-violet to red if sufficient protease activity is present. View the color change through the tube against a white sheet of paper.

Results obtained using various concentrations of protease

Concentration [mg/ml]	Color change within
2.0	about 1 minute
0.5	about 5 minutes
0.2	about 10 minutes

2.3. Parameters

Absorption

Spectral properties of the hydrolyzed substrate

Absorption (excitation) maximum in the
- neutral and alkaline range l = 574 nm, ε = 66,000 [l × mol^{-1} × cm^{-1}],
- in the acidic range l = 467 nm.

Chemical Name

Structural formula

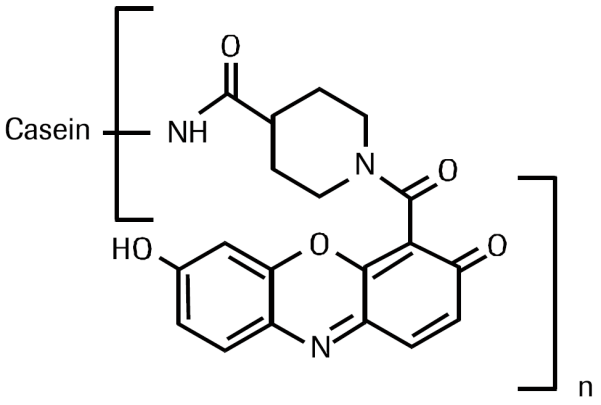

Fig. 1: Chemical structure of Universal Protease Substrate.

Emission

Spectral properties of the hydrolyzed substrate

Emission maximum in the neutral and alkaline range l = 584 nm, in the acidic range l = 559 nm.

3. Results

Results with different proteases

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

	Digestion by small amounts of proteases for 15 minutes (determination of the detection limit[1]		Digestion by large amounts of proteases overnight (maximum of total hydrolysis[1]	
Enzyme	**Enzyme amount**	**ΔOD_{574} nm**	**Enzyme amount**	**Absorbance ΔA_{574} 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

[1] The detection limit can be lowered by using fluorimetric analysis or by increasing the incubation time to, for example, overnight, see Figures 2 and 3.

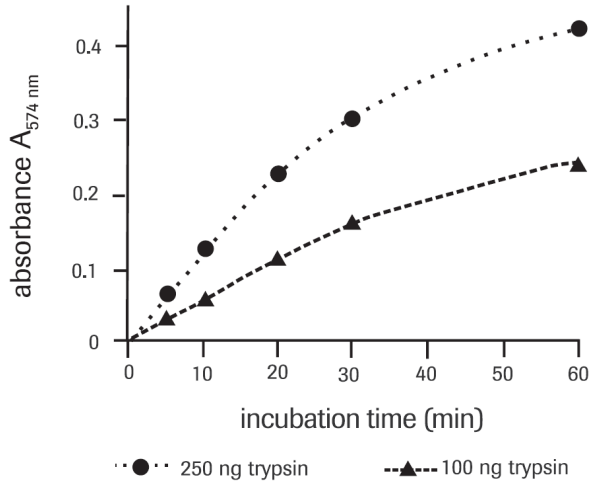

Fig. 2: Influence of the incubation time on the Casein-resorufin hydrolysis by trypsin.

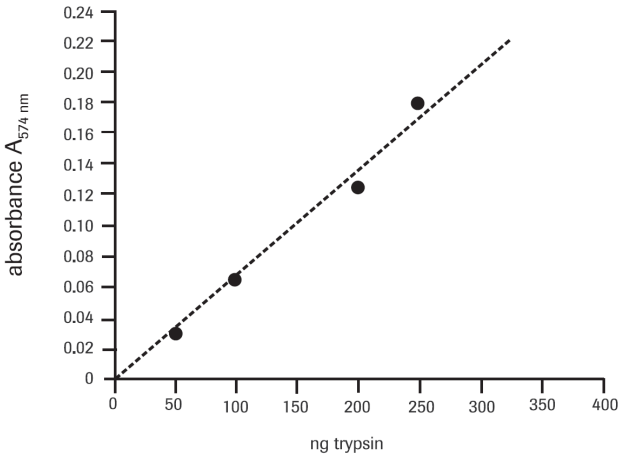

Fig. 3: Hydrolysis of Casein-resorufin by different amounts of trypsin.

4. Additional Information on this Product

4.1. Test 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.

Preparation

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

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

0621.11082469001 ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany