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Not for use in diagnostic procedures.



Chromozym TH

Tos-Gly-Pro-Arg-4-nitranilide acetate

 **Version: 19**

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Cat. No. 10 206 849 001 20 mg
Cat. No. 11 585 398 001 100 mg

Store the product at +15 to +25°C.

1.	General Information	3
1.1.	Contents	3
1.2.	Storage and Stability	3
	Storage Conditions (Product)	3
1.3.	Additional Equipment and Reagent required	3
1.4.	Application	3
2.	How to Use this Product	4
2.1.	Before you Begin	4
	Sample Materials	4
	General Considerations	4
	Calculation of thrombin activity	4
	Working Solution	4
2.2.	Protocols	5
	Thrombin assay	5
2.3.	Parameters	5
	Chemical Formula	5
	Chemical Name	5
	Contaminants	5
	Molecular Weight	5
	Purity	5
	Working Concentration	5
3.	Additional Information on this Product	6
3.1.	Test Principle	6
	Reaction principle	6
4.	Supplementary Information	7
4.1.	Conventions	7
4.2.	Changes to previous version	7
4.3.	Ordering Information	7
4.4.	Trademarks	8
4.5.	License Disclaimer	8
4.6.	Regulatory Disclaimer	8
4.7.	Safety Data Sheet	8
4.8.	Contact and Support	8

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Chromozym TH $C_{26}H_{35}N_8O_7SCOOCH_3$ - M_r 662.62	Powder	10 206 849 001	1 vial, 20 mg
			11 585 398 001	1 vial, 100 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	Chromozym TH	Store at +15 to +25°C.

1.3. Additional Equipment and Reagent required

For preparation of working solutions

- Tris* [2-amino-2-(hydroxymethyl)-1,3-propanediol]
- NaCl
- HCl, 2 M
- Bovine serum albumin* (BSA)
- Sodium azide
- Double-distilled water

1.4. Application

Chromozym TH is used as substrate for the determination of serine proteases, especially of thrombin, in aqueous solutions.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Thrombin solutions in the concentration range of approximately 0.06 U/ml.

⚠ Dilute immediately prior to the measurement with ice-cold buffer.

General Considerations

Calculation of thrombin activity

$$\text{U/ml sample solution} = \frac{V}{v \times \varepsilon \times d} \times \frac{\Delta A}{\text{min}}$$

Abbreviation	Description
V	Assay volume (3.2 ml)
v	Sample volume (0.1 ml)
ε 405 nm	10.4 [mmol ⁻¹ × l × cm ⁻¹] i Absorbance coefficient for 4-nitraniline.
d	Light path in the cuvette (1 cm)
$\Delta A/\text{min}$	Change in absorbance/minute

$$\text{U/ml sample solution} = \frac{3.2}{0.1 \times 10.4 \times 1} \times \frac{\Delta A}{\text{min}}$$

$$\text{U/ml sample solution} = 3.077 \times \frac{\Delta A}{\text{min}}$$

Working Solution

Solution No.	Solution	Composition/Preparation	Storage and Stability
1	Tris buffer	50 mM Tris, pH 8.3, 227 mM NaCl - Dissolve 605.5 mg Tris and 1.33 g NaCl in approximately 75 ml double-distilled water. - Adjust pH with 2 M HCl to 8.3; fill up to 100 ml with double-distilled water and dissolve 100 mg BSA. ⚠ Add 100 mg sodium azide as a preservative.	Store for at least 3 months at +2 to +8°C.
2	Chromozym TH	1.9 mM - Dissolve 5 mg Chromozym TH in 4 ml double-distilled water.	Store for at least 4 weeks at +2 to +8°C.

2.2. Protocols

Thrombin assay

Apply the following photospectrometer parameters:

Wavelength	Hg 405 nm
Light path	1 cm
Temperature	+25°C
Assay volume	3.2 ml
Measurement	against air

 See section, **Working Solution** for information on preparing solutions.

- 1 Pipette into a plastic cuvette:
 - 2.8 ml Tris buffer (Solution 1).
 - 0.3 ml Chromozym TH (Solution 2).

- 2 Mix and perform reaction at +25°C; check the temperature in the cuvette.

 **Do not start the reaction before the components are at +25°C.**

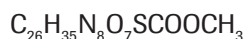
- 3 Start the reaction by adding 0.1 ml sample.

- 4 Mix, follow change in absorbance, and calculate $\Delta A/\text{minute}$ from the linear range.

 For the calculation of the thrombin activity, see section, **General considerations**.

2.3. Parameters

Chemical Formula



Chemical Name

Tos-gly-pro-arginine-4-nitranilide-acetate

Contaminants

1% free 4-nitraniline.

Molecular Weight

662.62 Da

Purity

90% Tos-Gly-Pro-Arg-4-nitranilide acetate (enzymatic).

Working Concentration

Approximately 0.2 mM.

3. Additional Information on this Product

3.1. Test Principle

Reaction principle

Assay for thrombin in aqueous solutions: Chromozym TH is cleaved by thrombin into a residual peptide and 4-nitraniline:



4-nitraniline is measured at 405 nm.

 *The absorbance difference per minute is used for the determination of the thrombin activity in U/ml.*

4. Supplementary Information

4.1. 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.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Bovine Serum Albumin	20 mg, 1 ml	10 711 454 001
Tris base	1 kg, <i>Not available in US</i>	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001

4. Supplementary Information

4.4. Trademarks

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

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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.

