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

Chromozym t-PA

N-Methylsulfonyl-D-Phe-Gly-Pro-Arg-4-nitranilide acetate

Version: 19
Content Version: December 2021

Cat. No. 11 093 037 001 20 mg

Store the product at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Chromozym t-PA N-Methylsulfonyl-D-Phe-Gly-Pro-Arg-4-nitranilide acetate	Powder	1 vial, 20 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 t-PA	Store at +15 to +25°C.

1.3. Additional Equipment and Reagent required

For procedure 1

- Tris* [2-amino-2-(hydroxymethyl)-1,3-propanediol]
- 1 M HCl
- Tween 80 (polyoxyethylenesorbitane monooleate)
- Citric acid-1-hydrate, A.R.I
- Autoclaved, double-distilled water

For procedure 2

- Plasmin from human plasma*
- Aprotinin*

1.4. Application

Chromozym t-PA is used as substrate for the determination of t-PA, both in purified preparations and in cell culture supernatants. It is also used to evaluate the content of one-chain and two-chain t-PA.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

t-PA solutions in the concentration range of 0.1 to 30 µg/ml.
⚠ ***Dilute higher concentrated t-PA solutions with Tris buffer.***

General Considerations

Procedure 1

Calculation of the t-PA activity in International Units (U/ml)

$$\text{t-PA (U/ml)} = \frac{\Delta A_{\text{sample/minute}} \times 1.6}{10.4 \times 0.1}$$

$$\text{t-PA (U/ml)} = \Delta A_{\text{sample/minute}} \times 1.54$$

If diluted samples are assayed, the dilution factor F must be taken into account:

$$\text{t-PA (U/ml)} = \Delta A_{\text{sample/minute}} \times 1.54 \times F$$

Calculation of the t-PA activity WHO Units (IU/ml)

For calculation in WHO Units, a t-PA standard must be run in parallel.

$$\text{t-PA (U/ml)} = \frac{\Delta A_{\text{sample/minute}} \times \text{IU/ml}_{\text{standard}}}{\Delta A_{\text{sample}}}$$

ℹ *One International Unit (U) t-PA ≈ approximately 32,500 WHO Units (IU) t-PA.*

Procedure 2

Calculation of the t-PA activity in International Units (U/ml)

$$\text{t-PA (U/ml)} = \frac{\Delta A_{\text{sample/minute}} \times 1.75}{10.4 \times 0.1}$$

$$\text{t-PA (U/ml)} = \Delta A_{\text{sample/minute}} \times 1.68$$

If diluted samples are assayed, the dilution factor F must be taken into account:

$$\text{t-PA (U/ml)} = \Delta A_{\text{sample/minute}} \times 1.68 \times F$$

ℹ *For calculation of the t-PA activity in WHO Units, see assay Procedure 1.*
ℹ *Concerning the determination of t-PA using plasminogen, refer to Lill H, 1987.*
ℹ *Concerning the determination of one- and two-chain t-PA in the same sample material with oligopeptide substrates some methods are described in Verheijen JH, et al, 1985.*

Working Solution

⚠ ***Use only plastic materials for storage, dilution, and the assay of t-PA. Do not use glass.***

Solution No.	Solution	Composition/Preparation	Storage and Stability
Procedure 1			
1	Tris buffer	▪ 100 mM Tris, pH 8.5, Tween 80, 0.15% (w/v) ▪ Dissolve 121 mg Tris with double-distilled water. Adjust to pH 8.5 with 1 M HCl. ▪ Separately dissolve 0.15 g Tween 80 with 10ml double-distilled water and add to the Tris solution. ▪ Fill up to 100 ml with double-distilled water.	Store for at least 2 weeks at +2 to +8°C.
2	Chromozym t-PA solution	▪ 4 mM ▪ Dissolve 5.1 mg Chromozym t-PA with 2 ml double-distilled water.	Store for at least 2 weeks at +2 to +8°C.
3	Citric acid solution	▪ 10% (w/v) ▪ Dissolve 10.94 g citric acid in 100 ml double-distilled water.	Store for at least 4 weeks at +2 to +8°C.
4	Reagent mixture	▪ Mix 9 parts Tris buffer (Solution 1) with 1 part Chromozym t-PA solution (Solution 2).	Store for 8 hours at +2 to +8°C or 4 hours at +15 to +25°C.
Procedure 2			
5	Plasmin solution	▪ 8 U/ml ▪ Dissolve 4 U Plasmin with 0.5 ml Tris buffer (Solution 1). ⓘ *The plasmin activity is determined at +25°C with Chromozym PL as substrate.*	Store in an ice bath until starting the assay. ⚠ ***Can be frozen in aliquots.***
6	Aprotinin solution	▪ 100 µg/ml ▪ Dissolve 5 mg Aprotinin with 50 ml double-distilled water.	Store for 1 week at +2 to +8°C. ⚠ ***Can be frozen.***

2.2. Protocols

Procedure 1

Apply the following photospectrometer parameters:

Wavelength	Hg 405 nm
Cuvettes	plastic
Light path	1 cm
Temperature	+37°C
Assay volume	1.6 ml
Measurement	against Reagent blank

⚠ ***Bring reagent mixture (Solution 4) to +37°C before starting the assay.***
⚠ ***Run at least one reagent blank per measuring series.***

Into a plastic cuvette, pipette:

Step	Reagent	t-PA sample measuring range		
		0.1 – 2.5 µg/ml	1 – 30 µg/ml	Reagent blank
1	t-PA sample	0.1 ml	0.1 ml	–
	Tris buffer (Solution 1)	–	–	0.1 ml
	Reagent mixture (Solution 2)	1.0 ml	1.0 ml	1.0 ml
2	Mix and incubate at +37°C for	30 min	3 min	3 or 30 min
3	Add Citric acid solution (Solution 3)	0.5 ml	0.5 ml	0.5 ml
4	Mix and read adsorbance of the sample against reagent blank (= ΔA_{sample})			

Procedure 2

Apply the following photospectrometer parameters:

Wavelength	Hg 405 nm
Cuvettes	plastic
Light path	1 cm
Temperature	+37°C
Assay volume	1.75 ml
Measurement	against Reagent blank

⚠ ***Bring Tris buffer (Solution 1) and Chromozym t-PA solution (Solution2) to +37°C before using the assay.***
⚠ ***Run at least one reagent blank per measuring series.***

Into a plastic cuvette pipette:

Step	Reagent	t-PA sample measuring range		
		0.1 – 2.5 µg/ml	1 – 30 µg/ml	Reagent blank
1	Tris buffer (Solution 1)	1.0 ml	1.0 ml	1.1 ml
	t-PA sample	0.1 ml	0.1 ml	–
	Plasmin solution (Solution 5)	0.025 ml	0.025 ml	0.025 ml
2	Mix and incubate at +37°C for 5 minutes			
3	Add Aprotinin solution (Solution 6)	0.025 ml	0.025 ml	0.025 ml
4	Mix and incubate at +37°C for 5 minutes			
5	Add Chromozym t-PA solution (Solution 2)	0.1 ml	0.1 ml	0.1 ml
6	Mix and incubate at +37°C for	30 min	3 min	3 or 30 min
7	Add Citric acid solution (Solution 3)	0.5 ml	0.5 ml	0.5 ml
8	Mix and read adsorbance of the sample against reagent blank (= ΔA_{sample})			

2.3. Parameters

Chemical Formula

$C_{24}H_{32}N_8O_7S \times CH_3COOH$

Chemical Name

N-Methylsulfonyl-D-Phe-Gly-Arg-4-nitranilide-acetate

Contaminants

<0.5% free 4-nitraniline.

Molecular Weight

636.7 Da

Purity

90% N-Methylsulfonyl-D-Phe-Gly-Arg-4-nitranilide acetate (enzymatic).

Working Concentration

Approximately 0.25 mM.

3. Additional Information on this Product

3.1. Test Principle

Reaction principle

Chromozym t-PA is cleaved by t-PA under formation of 4-nitraniline, which is measured at 405 nm. The t-PA activity is calculated in U/ml from the absorbance difference per minute, see section, **Protocols, Procedure 1**. However, it has to be considered that one-chain t-PA reacts less sensitive with oligopeptide substrated than two-chain t-PA.
t-PA in the sample can be measured in defined two-chain form after incubation with plasmin, see section, **Protocols, Procedure 2**:

CH_3-SO_2-D-Phe-Gly-Arg-4-nitranilide+ H_2O → CH_3-SO_2-D-Phe-Gly-Arg-OH + 4-nitraniline

3.2. References

- Lill H. Biochemische und physiologische Grundlagen zum Gewebe-Plasminogenaktivator (t-PA). Zeitschrift für die Gesamte Innere Medizin. 1987;42:478-486.
- Verheijen JH, de Jong YF, Chang Gaubius GTG. Quantitative analysis of the composition of mixtures of one-chain and two-chain tissue-type plasminogen activator with a spectrophotometric method. Thrombosis Research. 1985;3:281-288.

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.
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.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001
Plasmin, human	0.5 ml, 5 U	10 602 361 001
Aprotinin	10 mg	10 236 624 001
	50 mg	10 981 532 001
	100 mg	11 583 794 001

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 and select the corresponding product catalog.

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

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