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Not for use in diagnostic procedures.



# Chromozym PL

## Tosyl-Gly-Pro-Lys-4-nitranilide acetate

 **Version: 20**

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**Cat. No. 10 378 461 001**    20 mg

**Store the product at +15 to +25°C.**

|           |                                                     |          |
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# 1. General Information

## 1.1. Contents

| Vial / Bottle | Label                                                    | Function / Description | Content          |
|---------------|----------------------------------------------------------|------------------------|------------------|
| 1             | Chromozym PL<br>$C_{28}H_{35}N_6O_7SCOOCH_3 - M_r 634.7$ | Powder                 | 1 vial,<br>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 PL | 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
- Glycine
- Tween 20\*, 10% (w/v)
- PEG 6000
- Double-distilled water

## 1.4. Application

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

## 2. How to Use this Product

### 2.1. Before you Begin

#### Sample Materials

Plasmin solutions in the concentration range of approximately 0.2 to 0.3 U/ml.

**⚠ Dilute higher concentrated plasmin solutions with Sample dilution solution (Solution 5), see section, Working Solution.**

#### General Considerations

#### Calculation of plasmin activity

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

| Abbreviation                | Description                                                                                                             |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------|
| V                           | Assay volume (2.4 ml)                                                                                                   |
| v                           | Sample volume (0.2 ml)                                                                                                  |
| $\epsilon_{405 \text{ nm}}$ | 10.4 [ $\text{mmol}^{-1} \times \text{l} \times \text{cm}^{-1}$ ]<br><b>i</b> Absorbance coefficient for 4-nitraniline. |
| d                           | Light path in the cuvette (1 cm)                                                                                        |
| $\Delta A/\text{min}$       | Change in absorbance/minute                                                                                             |

$$\text{U/ml sample solution} = \frac{2.4}{0.2 \times 10.4 \times 1} \times \frac{\Delta A}{\text{min}}$$

$$\text{U/ml sample solution} = 1.15 \times \frac{\Delta A}{\text{min}}$$

#### Conversion factors

For Casein units at +37°C, WHO Units (British Standard 78/646), and International Units (U):

| A                   | 1 U =                                   |
|---------------------|-----------------------------------------|
| For plasmin, human  | 0.61 units (Casein)<br>1.83 units (WHO) |
| For plasmin, bovine | 0.65 units (Casein)<br>1.94 units (WHO) |

## Working Solution

Preparation of the solutions for approximately 50 determinations.

| Solution No. | Solution                 | Composition/Preparation                                                                                                                                                                                                                       | Storage and Stability                                                                  |
|--------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| 1            | Tris buffer              | <ul style="list-style-type: none"> <li>50 mM Tris, pH 8.2</li> <li>Dissolve 605.5 mg Tris and 584 mg NaCl in double-distilled water.</li> <li>Adjust pH with 2 M HCl to 8.2 and fill up to 100 ml with double-distilled water.</li> </ul>     | Store for at least 2 weeks at +2 to +8°C.<br><b>⚠️ Avoid contamination with germs.</b> |
| 2            | NaCl solution            | <ul style="list-style-type: none"> <li>0.9%</li> <li>Dissolve 900 mg NaCl in 100 ml double-distilled water.</li> </ul>                                                                                                                        | Store for several months at +15 to +25°C.                                              |
| 3            | Glycine solution         | <ul style="list-style-type: none"> <li>100 mM</li> <li>Dissolve 75 mg glycine in 10 ml double-distilled water.</li> <li>Add 200 µl Tween 20, ready-to-use solution.</li> <li><b>i</b> Final concentration of Tween 20 is 0.2% w/v.</li> </ul> | Store for at least 2 weeks at +2 to +8°C.<br><b>⚠️ Avoid contamination with germs.</b> |
| 4            | Chromozym PL             | <ul style="list-style-type: none"> <li>3 mM</li> <li>Dissolve 9.5 mg Chromozym PL in 5 ml glycine solution (Solution 3).</li> </ul>                                                                                                           |                                                                                        |
| 5            | Sample dilution solution | <ul style="list-style-type: none"> <li>pH 2.5</li> <li>Dissolve 500 mg PEG 6000 and 375 mg glycine in double-distilled water, adjust to a pH value of 2.5 with 2 M HCl, and fill up to 100 ml with double-distilled water.</li> </ul>         |                                                                                        |

## 2.2. Protocols

### Plasmin assay

Apply the following photospectrometer parameters:

|              |             |
|--------------|-------------|
| Wavelength   | Hg 405 nm   |
| Light path   | 1 cm        |
| Temperature  | +25°C       |
| Assay volume | 2.4 ml      |
| Measurement  | against air |

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

- 1 Pipette into a plastic cuvette:
  - 1.6 ml Tris buffer (Solution 1).
  - 0.2 ml NaCl (Solution 2).
  - 0.4 ml Chromozym PL (Solution 4).

- 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 0.2 ml sample.

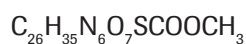
- 4 Mix, follow change in absorbance every 30 seconds, and calculate  $\Delta A/\text{minute}$  from the linear range.

**i** For the calculation of the plasmin activity, see section, **General Considerations**.

### 3. Additional Information on this Product

## 2.3. Parameters

### Chemical Formula



### Chemical Name

Tosyl-glycyl-poly(l-lysine-4-nitranilide)-acetate

### Contaminants

<0.5% free 4-nitraniline.

### Molecular Weight

634.7 Da

### Purity

90% Tosyl-Gly-Pro-Lys-4-nitranilide acetate (enzymatic).

### Working Concentration

Approximately 0.3 to 0.6 mM.

## 3. Additional Information on this Product

### 3.1. Test Principle

#### Reaction principle

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



4-nitraniline is measured at 405 nm.

**i** The absorbance difference per minute is used for the determination of the plasmin 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 |                                  |                |
| Tween 20       | 50 ml, 5 x 10 ml                 | 11 332 465 001 |
| 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.

