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Not for use in diagnostic procedures.



# Chromozym PK

## Benzoyl-Pro-Phe-Arg-4-nitranilide acetate

 **Version: 17**  
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**Cat. No. 10 378 445 001** 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 PK $C_{33}H_{39}N_8O_6COOCH_3$ - $M_r$ 702.9	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 PK	Store at +15 to +25°C.

## 1.3. Additional Equipment and Reagent required

### For preparation of working solutions

- Dextran sulfate
- 1 M HCl
- Tris\* [2-Amino-2-(hydroxymethyl)-1.3-propanediol]
- Imidazole
- NaCl
- Double-distilled water

### For plasma kallikrein assay

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

- Plastic cuvettes
- Cuvette rack with thermostat
- 0.1 1 M sodium citrate solution

## 1.4. Application

Chromozym PK is used as substrate for the determination of serine proteases, especially of plasma kallikrein, such as in citrated plasma.

## 2. How to Use this Product

### 2.1. Before you Begin

#### General Considerations

##### Calculation of plasma kallikrein activity

$$\text{U/ml citrated plasma} = \frac{V}{v \times \epsilon \times d} \times F \times \frac{\Delta A}{\Delta t}$$

Abbreviation	Description
F	Dilution factor = 2
V	Assay volume (2.022 ml)
v	Sample volume (0.02 ml)
$\epsilon_{405 \text{ nm}}$	10.4 [ $\text{mmol}^{-1} \times \text{l} \times \text{cm}^{-1}$ ] <i>i</i> Absorbance coefficient for 4-nitraniline.
d	Light path in the cuvette (1 cm)
$\Delta A/\Delta t = \Delta A/\text{min}$	Change in absorbance/minute

$$\text{U/ml citrated sample} = \frac{2.02}{0.02 \times 10.4 \times 1} \times 2 \times \frac{\Delta A}{\text{min}}$$

#### Working Solution

Preparation of the solutions for approximately 50 determinations.

Solution No.	Solution	Composition/Preparation	Storage and Stability
1	Dextran sulfate solution	<ul style="list-style-type: none"> <li>25 mg/ml dextran sulfate</li> <li>Dissolve 2.5 g dextran sulfate 500, sodium salt, in double-distilled water and fill up to 100 ml.</li> </ul>	Store for at least 4 weeks at +2 to +8°C. <b>⚠ Keep at 0°C before use in the assay.</b>
2	HCl	1.0 M HCl solution	Store for several months at +15 to +25°C.
3	Tris/imidazole buffer	15 mM, pH 7.9; ion strength 0.15 <b>a) 0.25 M Tris, 0.25 M imidazole:</b> Dissolve 3.03 g Tris and 1.70 g imidazole in 50 ml 1 M HCl (Solution 2) and fill up to 100 ml with double-distilled water. <b>b) 0.33 M Tris, 0.33 M imidazole, 0.33 M NaCl:</b> Dissolve 4.04 g Tris, 2.27 g imidazole, and 1.95 g NaCl in approximately 75 ml double-distilled water; fill up to 100 ml with double-distilled water. <b>c) NaCl solution (2 M NaCl):</b> Dissolve 11.63 g NaCl in approximately 75 ml double-distilled water and fill up to 100 ml.	Store for at least 4 weeks at +2 to +8°C. <b>⚠ Avoid contamination with germs.</b>
4	Incubation buffer	<ul style="list-style-type: none"> <li>Mix Solutions 3a and 3b to reach a resulting buffer of pH 7.9.</li> <li>Mix 5 ml of this buffer with 5 ml of Solution 3c.</li> </ul> <b>⚠ Dilute this buffer concentrate 1 + 9 immediately before use.</b>	<b>⚠ Dilute freshly and warm up to +37°C before use in the assay.</b>

5	Chromozym PK solution <ul style="list-style-type: none"> <li>▪ 1.3 mM</li> <li>▪ Dissolve 50 mg Chromozym PK in 50 ml double-distilled water.</li> </ul>	Store for at least 4 weeks at +2 to +8°C. <b>⚠ Warm up to +37°C before use in the assay.</b>
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## 2.2. Protocols

### Preparation of sample material

- 1 Remove blood from an uncongested vein.
  - 2 Immediately after removal, mix 9 volume parts of blood with 1 volume part 0.1 M sodium citrate solution.
  - 3 Centrifuge the sample at approximately 2,000 × *g* (approximately 3,000 rpm with conventional lab centrifuges) for 10 minutes within 2 hours after removal.
- i* Citrated plasma is stable for up to 4 hours stored at +2 to +8°C, or for 6 months stored at –15 to –25°C.

### Plasma kallikrein assay

Apply the following photospectrometer parameters:

Wavelength	Hg 405 nm
Cuvettes	plastic <i>i</i> Use a cuvette rack with thermostat.
Light path	1 cm
Temperature	+37°C
Assay volume	2.02 ml
Measurement	against air

**⚠ Equilibrate Solutions 4 and 5 to +37°C before using in the assay. Keep Solution 1 at 0°C.**

- i* A reagent blank is not necessary.  
*i* See section, **Working Solution** for information on preparing solutions.
- 1 For the Activation, pipette into a plastic cuvette:
    - 0.1 ml plasma
    - 0.1 ml Dextran sulfate solution (Solution 1).
  - 2 Mix and incubate for 7 minutes at 0°C (= activated sample).
  - 3 For the Kallikrein catalyzed reaction, pipette into a plastic cuvette:
    - 1.52 ml Incubation buffer (Solution 4).
    - 0.52 ml Chromozym PK solution (Solution 5).
    - 0.02 ml activated sample (from Step 1).
  - 4 Mix immediately.
    - Read absorption within 30 seconds and simultaneously start the stop watch.
    - Repeat reading after 1, 2, 3, and 4 minutes or follow the reaction by a recorder.

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

## 2. How to Use this Product

### 2.3. Parameters

#### Chemical Formula

$C_{33}H_{39}N_8O_6COOCH_3$

#### Chemical Name

Benzoyl-prolyl-phenalanyl-arginine-4-nitranilide acetate

#### Contaminants

<0.5% free 4-nitraniline.

#### Molecular Weight

702.9 Da

#### Purity

90% Benzoyl-Pro-Phe-Arg-4-nitranilide acetate (enzymatic).

#### Working Concentration

Approximately 0.5 mM.

## 3. Additional Information on this Product

### 3.1. Test Principle

#### Reaction principle

Chromozym PK is cleaved by plasma kallikrein into a residual peptide and free 4-nitraniline which is measured at 405 nm.

**i** *The absorbance difference per minute is used for the determination of the kallikrein activity in U/ml:*

Starting material	reacts with	to
Factor XII	dextran sulfate	Factor XIIa
Plasma prokallikrein	factor XIIa	Plasma-kallikrein
Bz-Pro-Phe-Arg-4-NA + H <sub>2</sub> O	plasma-kallikrein	B-Pro-Phe-Arg + 4-nitraniline

## 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

**i** *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		
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



## 4.4. 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.

