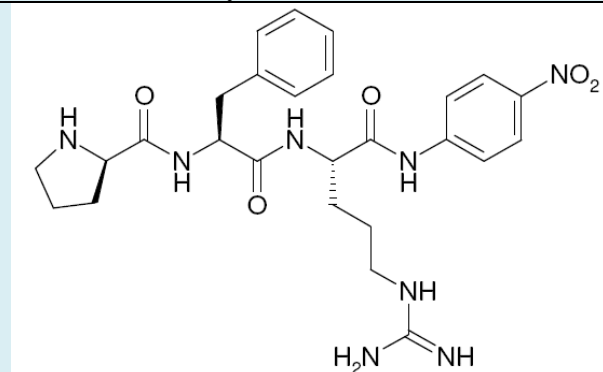
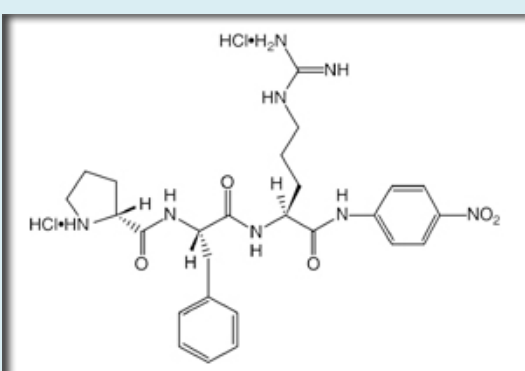


**COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF KALLICREIN
CHROMOGENIC SUBSTRATE (HYPHEN BioMed CS-31(02))**

	HYPHEN BioMed	Chromogenix								
Product name	BIOPHEN CS-31(02)	S2302								
Product reference	A229031	82 03 40								
Specificity	Recommended substrate for Kallikrein.	Chromogenic substrate for plasma kallikrein, FXIa and FXIIa.								
Peptide sequence	H-D-Pro-Phe-Arg-pNa, 2HCl	H-D-Pro-Phe-Arg-pNA·2HCl								
Developed name	H-D-Prolyl-L-phenylalanyl-L-arginine-para-nitroaniline, -dihydrochloride	H-D-Prolyl-L-phenylalanyl-Larginine- p-nitroaniline dihydrochloride.								
Chemical structure	 <p align="center"><chem>C26H34N8O5, 2HCl</chem></p>									
Proposed presentation	25mg	25 mg								
Molarity	~46 μ M	-								
Bulking agents	Mannitol	Mannitol (60 mg/vial)								
Purity grade	> 95%	-								
Solubility	\geq 5mg/ml in H ₂ O	> 10 mmol/l in H ₂ O								
Molecular weight	538.6 Da (basic structure)	611.6 Da* (*2HCl included)								
Free pNA content	< 0.05%	NA								
E316 nm:	NA	$1.27 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$								
Respective reactivities	<table border="1"> <thead> <tr> <th>Kallikrein</th> <th>FXa</th> <th>Thrombin</th> <th>aPC</th> </tr> </thead> <tbody> <tr> <td align="center">100</td> <td align="center">10</td> <td align="center">3</td> <td align="center">5</td> </tr> </tbody> </table> <p>Assay conditions must be established for making the substrate totally specific for Kallikrein.</p>	Kallikrein	FXa	Thrombin	aPC	100	10	3	5	
Kallikrein	FXa	Thrombin	aPC							
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Stability of the lyophilized product	Until the expiration date printed on the vial. (30 months at 2-8 °C from the manufacturing date)	Stable until expiry date if stored at 2-8°C. Avoid exposure to light. The substance is hygroscopic and should be stored dry.								
Stability of the reconstituted product	- 7 days at room temperature (18-25 °C) - 3 months at 2-8 °C - Do not freeze.	4 mmol/l in H ₂ O is stable for more than 6 months at 2-8°C.								

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Suitable stock solution	According to the research protocol used, the BIOPHEN 31(02) chromogenic substrate can be restored with variable volumes of distilled water ; for example 5 mL can be used for a substrate concentration of 5 mg/mL, or 20 mL for a substrate concentration of 1.25 mg/mL.	4 mmol/l in H ₂ O.																				
Kinetic data	Same characteristics	Human plasma kallikrein: Km= 2 . 10 ⁻⁴ mol/l, V = 6.8 . 10 ⁻⁶ mol/min . PEU. Determined at 37°C in 2.5 ml of 0.05 mol/l Tris buffer pH 7.8 I 0.05. PEU (Plasma Equivalent Units). PEU refers to the activity generated from 1 ml of normal human plasma using Cephotes® (NYCO, Oslo) as activator. The same Km was obtained for a highly purified human plasma kallikrein.																				
Applications	<p>For in vitro use only. All research studies and protocols where a source of chromogenic substrate for Kallikrein is required.</p> <p>Suggested protocol:</p> <table border="1" data-bbox="352 1043 820 1700"> <thead> <tr> <th>Reagent</th> <th>Water bath</th> </tr> </thead> <tbody> <tr> <td>Prekallikrein pool (=C)</td> <td>200µL</td> </tr> <tr> <td>FXIIa at 1µg/ml or serial dilutions (in TBSA buffer)</td> <td>200µL</td> </tr> <tr> <td colspan="2">Mix and incubate for 5 min at 37°C</td> </tr> <tr> <td>Tris 0.05M,NaCl 0.15M, pH 8.00 buffer</td> <td>200 µL</td> </tr> <tr> <td colspan="2">Mix and incubate for 2 min at 37°C</td> </tr> <tr> <td>Substrate (reconstituted at 2.5mg/ml in distilled water)</td> <td>200µl</td> </tr> <tr> <td colspan="2">Mix and incubate for 2 min at 37°C</td> </tr> <tr> <td>Citric acid 2%</td> <td>300µl</td> </tr> <tr> <td colspan="2">Read A405nm against the sample blank.</td> </tr> </tbody> </table>	Reagent	Water bath	Prekallikrein pool (=C)	200µL	FXIIa at 1µg/ml or serial dilutions (in TBSA buffer)	200µL	Mix and incubate for 5 min at 37°C		Tris 0.05M,NaCl 0.15M, pH 8.00 buffer	200 µL	Mix and incubate for 2 min at 37°C		Substrate (reconstituted at 2.5mg/ml in distilled water)	200µl	Mix and incubate for 2 min at 37°C		Citric acid 2%	300µl	Read A405nm against the sample blank.		<p>The substrate has been used for the determination of:</p> <ol style="list-style-type: none"> 1. Prekallikrein in plasma) 2. Kallikrein inhibitors in plasma 3. F XII in plasma 4. Kallikrein-like activity in plasma 5. Prekallikrein activator in albumin and immunoglobulin preparations
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