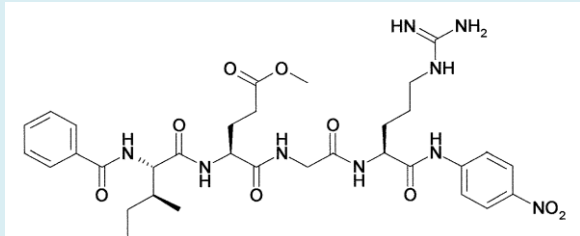
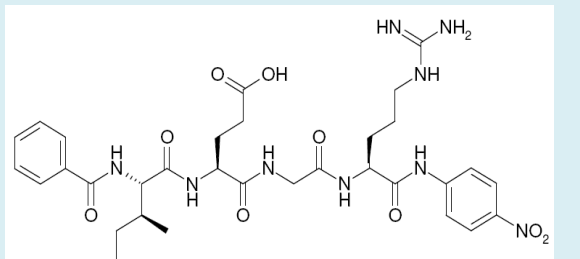
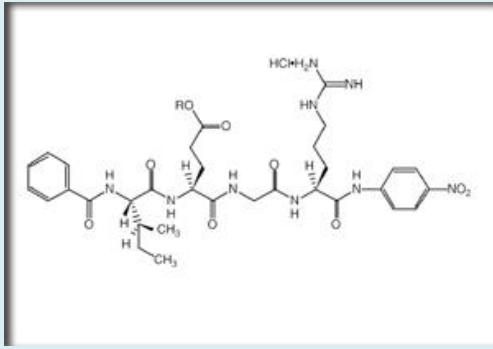


## COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF FXa CHROMOGENIC SUBSTRATE (HYPHEN BioMed CS-11(22))

	<b>HYPHEN BioMed</b>	<b>Chromogenix</b>
Product name	<b>BIOPHEN CS-11(22)</b>	<b>S-2222</b>
Product reference	A229015	82 03 16
Specificity	Recommended substrate for Factor Xa.	Chromogenic substrate for FXa.
Peptide sequence	Mixture (50%-50%) of Bz-Ile-Glu( $\gamma$ OCH <sub>3</sub> )-Gly-Arg-pNa, (HCl) (form 1) and Bz-Ile-Glu ( $\gamma$ OH)Gly-Arg-pNa, (HCl) (form 2)	Bz-Ile-Glu( $\gamma$ -OR)-Gly-Arg-pNA·HCl R=H (50%) and R=CH <sub>3</sub> (50%)
Developed name	Benzoyl-L-isoleucyl-L-(gamma methyl ester)glutamyl-glycyl-L-arginine-para-nitroaniline (hydrochloride) (form 1) and Benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginine-para-nitroaniline (hydrochloride) (form 2)	N-Benzoyl-L-isoleucyl-L-glutamylglycyl-L-arginine-p-nitroaniline hydrochloride and its methyl ester
Chemical structure	<p>Form 1 :</p>  <p>C33H45N9O9 (HCl)</p> <p>-----</p> <p>Form 2 :</p>  <p>C32H43N9O9 (HCl)</p>	
Proposed presentation	25 mg	25 mg
Molarity	~35 $\mu$ mol / vial	
Bulking agents	Mannitol	Mannitol (120mg/vial)
Purity grade	> 95%	NA
Solubility	$\geq$ 5 mg/mL in distilled water	6 mmol/L in H <sub>2</sub> O 2 mmol/L in Tris buffer (pH 8.3, I 0.25)
Molecular weight	711.8 Da (form 1) and 697.7 Da (form 2) (basic structure)	748.3* (R = CH <sub>3</sub> ) and 734.3* (R = H) (* HCl included)
Free pNA content	<0.05%	NA
E316 nm:	NA	$1.27 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$



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CHROMOGENIC SUBSTRATE (HYPHEN BioMed CS-11(22))**

Respective reactivities	<table border="1"> <tr> <td>FXa</td> <td>Thrombin</td> <td>Plasmin</td> <td>Kallicrein</td> <td>aPC</td> </tr> <tr> <td>100</td> <td>1</td> <td>2</td> <td>3</td> <td>0</td> </tr> </table>	FXa	Thrombin	Plasmin	Kallicrein	aPC	100	1	2	3	0	very sensitive to trypsin
	FXa	Thrombin	Plasmin	Kallicrein	aPC							
100	1	2	3	0								
Assay conditions must be duly established for rendering the assay conditions totally specific for Factor Xa, when this substrate is used.												
Stability of the lyophilized product	Until the expiration date printed on the vial. (30 months at 2-8°C from the manufacturing date)	Stable at 2-8°C for more than 3 years. The substance is somewhat hygroscopic and should be stored dry.										
Stability of the reconstituted product	<ul style="list-style-type: none"> <li>7 days at room temperature (18-25 °C)</li> <li>- 3 months at 2-8 °C</li> <li>- <b>Do not freeze.</b></li> </ul>	4 mmol/L in H <sub>2</sub> O is stable for at least 6 months at 2 to 8°C.										
Suitable stock solution	According to the research protocol used, the BIOPHEN CS-11(22) chromogenic substrate can be restored with variable volumes of distilled water ; for example 10 mL can be used for a substrate concentration of 2.5 mg/mL, or 20 mL for a substrate concentration of 1.25 mg/mL. Shake thoroughly until complete dissolution (vortex). Let to stabilize for 30 min. at room temperature.	1-4 mmol/L in H <sub>2</sub> O. Vigorous haking or an ultrasonic bath is recommended for dissolution, which is slow.										
Kinetic data	Same characteristics	Factor Xa (bovine): Km=3 . 10-4 mol/L. kcat=100 sec <sup>-1</sup> in 37°C Tris buffer pH 8.3, I0.2 Trypsin (porcine): Km=2 . 10-5 mol/L, kcat=280 sec <sup>-1</sup> in 37°C Tris buffer pH9.0, I 0.25										
Applications	For in vitro use only. All research studies and protocols where a source of chromogenic substrate for Factor Xa is required.											
	Suggested protocol:											
	Reagent	Water bath										
	Tris 0.05M,NaCl 0.30M, pH 8.40 buffer	400 µL										
	Human or Bovine FXa from 2.50µg/ml (=C) or serial dilutions, or plasma sample	100 µL										
	Mix and incubate for 1 min at 37 °C											
	Substrate (reconstituted at 2.5mg/ml in distilled water)	100µl										
	Mix and incubate for 3 min at 37 °C											
	Citric acid 2%	300µl										
Read A405nm against the sample blank.												
The substrate has been used for the determination of: <ol style="list-style-type: none"> <li>1. FX in plasma</li> <li>2. FXa in plasma</li> <li>3. FXa inhibitor in plasma</li> <li>4. Heparin in plasma</li> <li>5. Factor VIII in plasma</li> <li>6. Coagulating enzyme from horseshoe crab</li> <li>7. Trypsin in duodenal fluid</li> </ol>												