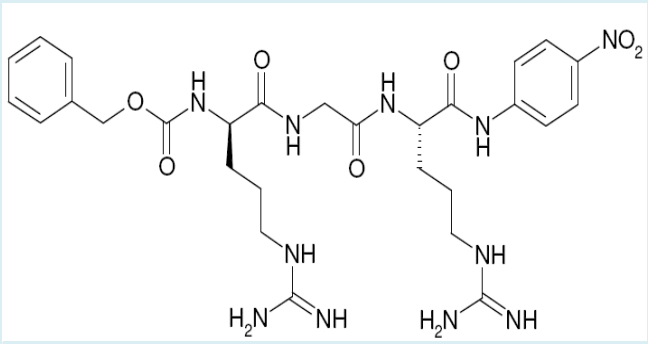
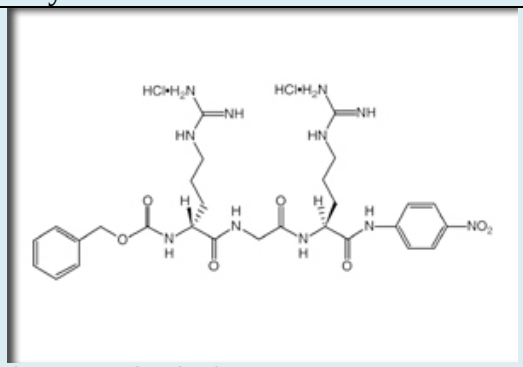
COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF FXa CHROMOGENIC SUBSTRATE (HYPHEN BioMed CS-11(65))

	HYPHEN BioMed	**Chromogenix**
Product name	**BIOPHEN CS-11(65)**	**S2765**
Product reference	A229014 / A229114	82 14 13
Specificity	Recommended substrate for Factor Xa.	Chromogenic substrate for FXa.
Peptide sequence	Z-D-Arg-Gly-Arg-pNA, 2HCl	Z-D-Arg-Gly-Arg-pNA · 2HCl
Developed name	Benzyloxycarbonyl-D-arginyl-glycyl-L-arginine-para-nitroaniline, -dihydrochloride	N-α-Benzyloxycarbonyl-Darginyl-L-glycyl-L-arginine-pnitroaniline-dihydrochloride
Chemical structure	 $C_{28}H_{39}N_{11}O_7$, 2HCl	 $C_{28}H_{39}N_{11}O_7$, 2HCl
Proposed presentation	• 25 mg (#229014) • 100 mg (#229114)	25 mg
Molarity	A229014 : ~39µM A229114 : ~156µM	-
Bulking agents	Mannitol	Mannitol (60mg/vial)
Purity grade	> 95%	-
Solubility	≥ **5 mg/mL in H2O or Tris buffer**	> 40 mmol/L in H2O > 10 mmol/L in Tris buffer (pH 8.3, I 0.25)
Molecular weight	641.7 Da (basic structure)	714.6 Da* (*2HCl included)
Free pNA content	< 0.05%	NA
E316 nm:	NA	$1.27 \cdot 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$
Respective reactivities	**FXa**: 100; **Thrombin**: 1; **Plasmin**: 1; **Kallicrein**: 15; **aPC**: 2 **Assay conditions must be duly established for rendering the assay conditions totally specific for Factor Xa, when this substrate is used.**	very sensitive to trypsin

Aniara 6560 Gove Court Mason, OH 45040 **Phone**: 513-770-1991 **Toll Free**: 866-783-3797 **FAX**: 513-573-9241 **Web**: www.aniara.com **Email**: info@aniara.com

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<table>
<tr><td>Stability of the lyophilized product</td><td>Until the expiration date printed on the vial.
(30 months at 2-8°C from the manufacturing date)</td><td>2-8°C until the expiration date.
The substance is hygroscopic and should be stored in a dry place.</td></tr>
<tr><td>Stability of the reconstituted product</td><td>- 7 days at room temperature (18-25°C)
- 3 months at 2-8 °C
- Do not freeze.</td><td>2 mmol/L in H2O is stable for six months at 2 to 8°C</td></tr>
<tr><td>Suitable stock solution</td><td>A229014: According to the research protocol used, the BIOPHEN CS-11(65) 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 (3.9 µM), or 20 mL for a substrate concentration of 1.25 mg/mL (1.95 µM). Shake thoroughly until complete dissolution (vortex). Let to stabilize for 30 min. at room temperature.
A229114: According to the research protocol used, the BIOPHEN CS-11(65) chromogenic substrate can be restored with variable volumes of distilled water ; for example 40 mL can be used for a substrate concentration of 2.5 mg/mL (3.9 µM), or 80 mL for a substrate concentration of 1.25 mg/mL (1.95 µM). Shake thoroughly until complete dissolution (vortex). Let to stabilize for 30 min. at room temperature.</td><td>1-2 mmol/L in H2O.</td></tr>
<tr><td>Kinetic data</td><td>Same characteristics.</td><td>Factor Xa (bovine): km=1. 10-4 mol/L, kcat=290 sec-1in Tris buffer pH 8.3, I 0.25 at 37°C.
Factor Xa: (human plasma activated with Russel's Viper Venom): km=3 . 10-4 mol/L in Tris buffer pH 7.8, I 0.4 at 37°C.</td></tr>
<tr><td>Applications</td><td>For in vitro use only.
All research studies and protocols where a source of chromogenic substrate for Factor Xa is required.
Suggested protocol:
<table>
<tr><th>Reagent</th><th>Water bath</th></tr>
<tr><td>Tris0.05M,NaCl0.30M, pH 8.40 buffer</td><td>400 µL</td></tr>
<tr><td>Human or Bovine FXa from 2.50µg/ml (=C), or serial dilutions, or plasma sample</td><td>100 µL</td></tr>
<tr><td colspan="2">Mix and incubate for 1 min at 37°C</td></tr>
<tr><td>Substrate (reconstituted at 2.5mg/ml in distilled water)</td><td>100µl</td></tr>
<tr><td colspan="2">Mix and incubate for 3 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>
</table></td><td></td></tr>
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