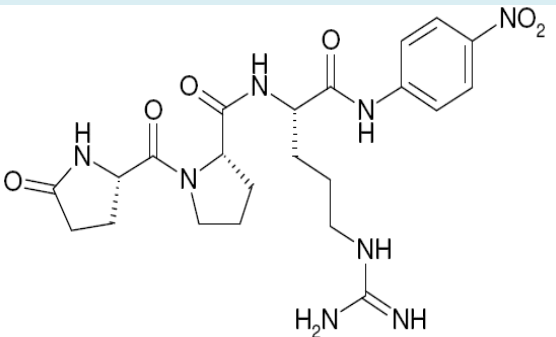
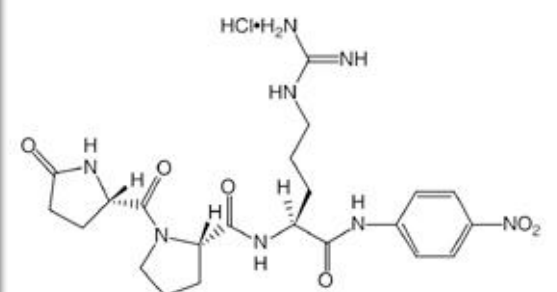


## COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF ACTIVATED PROTEIN C (aPC) CHROMOGENIC SUBSTRATE (HYPHEN BioMed CS-21(66))

	<b>HYPHEN BioMed</b>	<b>Chromogenix</b>										
Product name	<b>BIOPHEN CS-21(66)</b>	<b>S-2366</b>										
Product reference	A229021	82 10 90										
Specificity	Recommended substrate for Activated Protein C.(SaPC-21)	Chromogenic substrate for activated protein C and FXIa.										
Peptide sequence	p-Glu-Pro-Arg-pNa. HCl	pyroGlu-Pro-Arg-pNA-HCl										
Developed name	L-Pyroglutamyl-L-prolyl-L-arginine-para-nitroaniline, -hydrochloride	L-Pyroglutamyl-L-prolyl-L-arginine p-Nitroaniline hydrochloride.										
Chemical structure	 <p><chem>C22H30N8O6</chem>, HCl</p>											
Proposed presentation	25mg	25 mg										
Molarity	≈ 50 μmol/vial	-										
Bulking agents	Mannitol	Mannitol (40 mg/vial)										
Purity grade	> 95%	-										
Solubility	≥ 5 mg/mL in H2O	> 10 mmol/L in H2O										
Molecular weight	502.5 (basic structure)	539.0* (* HCl included)										
Free pNA content	< 0.05%	NA										
E316 nm:	NA	$1.27 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$										
Respective reactivities	<table border="1" data-bbox="351 1758 885 1848"> <thead> <tr> <th>APC</th> <th>FXa</th> <th>Plasmin</th> <th>Kallicrein</th> <th>Thrombin</th> </tr> </thead> <tbody> <tr> <td>100</td> <td>7</td> <td>85</td> <td>150</td> <td>125</td> </tr> </tbody> </table> <p>Assay conditions must be established for making the substrate totally specific for activated protein C.</p>	APC	FXa	Plasmin	Kallicrein	Thrombin	100	7	85	150	125	also readily split by trypsin, thrombin, plasmin and tissue plasminogen activator . It is split by FXIIa, plasma kallikrein and FXa as well.
APC	FXa	Plasmin	Kallicrein	Thrombin								
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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.										



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Stability of the reconstituted product	-7 days at room temperature (18-25 °C) - 3 months at 2-8 °C - <b>Do not freeze.</b>	2 mmol/L in H <sub>2</sub> O is stable for more than 6 months at 2 to 8°C																
Suitable stock solution	According to the research protocol used, the BIOPHEN CS-21(66) 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.	2-3 mmol/L in H <sub>2</sub> O.																
Kinetic data	Same characteristics	Protein C: Km=2 . 10 <sup>-4</sup> mol/L and kcat=80 sec <sup>-1</sup> (The enzyme is assumed to be pure. Mol. wt. 62 000) Determined with RVV activated bovine Protein C in 0.05 mol/L Tris, pH 8.0, I 0.25 (NaCl) and 4mmol/L CaCl <sub>2</sub> at 37°C. Km=8 . 10 <sup>-4</sup> mol/L and kcat=160 sec <sup>-1</sup> . Determined with thrombintrombomodulin complex activated human Protein C in 0.05 mol/L Tris, pH 8.0, I 0.13 (NaCl) and 10 mmol/L CaCl <sub>2</sub> at 25°C .  FXIa: Km=4 . 10 <sup>-4</sup> mol/L and kcat E 1000 sec <sup>-1</sup> in 0.1 mol/L Phosphate buffer, pH 7.6, I 0.15 mol/L (NaCl) at 37°C. Km=5.6 . 10 <sup>-4</sup> mol/L and kcat= 350 sec <sup>-1</sup> in 0.09 mol/L Tris, pH 8.3, 0.09 mol/L NaCl, 1 mg/mL of bovine serum albumin at room temperature.																
Applications	For in vitro use only. All research studies and protocols where a source of chromogenic substrate for Activated Protein C is required.  Suggested protocol: <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">Reagent</td> <td style="width: 50%;">Water bath</td> </tr> <tr> <td>Tris 0.05M, CsCl 0.26M, CaCl<sub>2</sub> 0.004M, pH 8.30 buffer</td> <td>400 µL</td> </tr> <tr> <td>Human aPC from 2.50µg/ml (=C) or serial dilutions in TBSA buffer, 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 5 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>	Reagent	Water bath	Tris 0.05M, CsCl 0.26M, CaCl <sub>2</sub> 0.004M, pH 8.30 buffer	400 µL	Human aPC from 2.50µg/ml (=C) or serial dilutions in TBSA buffer, 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 5 min at 37 °C		Citric acid 2%	300µl	Read A405nm against the sample blank.		
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