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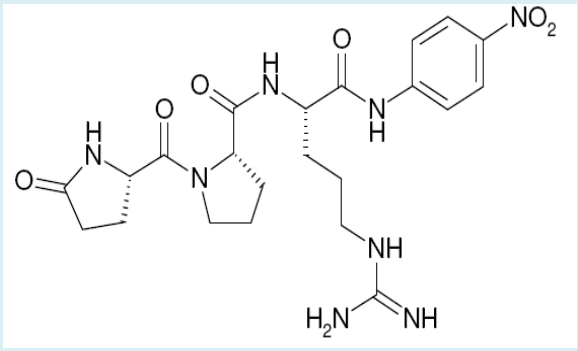
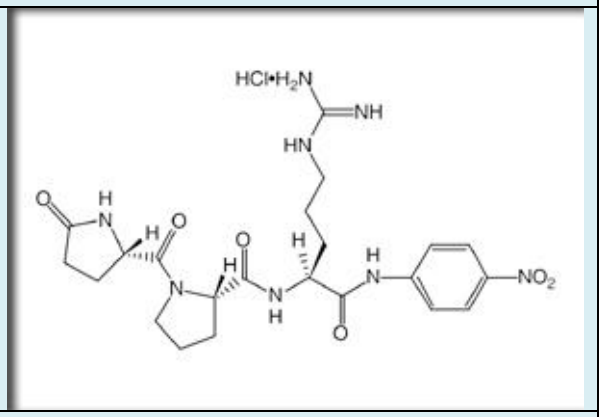
Manufactured By: HYPHEN BioMed

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

	HYPHEN BioMed	**Chromogenix**
Product name	**BIOPHEN CS-21(66)**	**S-2366**
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	 $C_{22}H_{30}N_8O_6$, HCl	
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 .10^4 mol^{-1} . L . cm^{-1}
Respective reactivities	APC: 100; FXa: 7; Plasmin: 85; Kallicrein: 150; Thrombin: 125 Assay conditions must be established for making the substrate totally specific for activated protein C.	also readily split by trypsin, thrombin, plasmin and tissue plasminogen activator . It is split by FXIIa, plasma kallikrein and FXa as well.
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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<table>
<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 more than 6 months at 2 to 8°C</td></tr>
<tr><td>Suitable stock solution</td><td>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.</td><td>2-3 mmol/L in H2O.</td></tr>
<tr><td>Kinetic data</td><td>Same characteristics</td><td>Protein C: Km=2 . 10-4 mol/L and kcat=80 sec-1 (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 CaCl2 at 37°C.
Km=8 . 10-4 mol/L and kcat=160 sec-1. 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 CaCl2 at 25°C .

FXIa: Km=4 . 10-4 mol/L and kcat E 1000 sec-1 in 0.1 mol/L Phosphate buffer, pH 7.6, I 0.15 mol/L (NaCl) at 37°C.
Km=5.6 . 10-4 mol/L and kcat= 350 sec-1 in 0.09 mol/L Tris, pH 8.3, 0.09 mol/L NaCl, 1 mg/mL of bovine serum albumin at room temperature.</td></tr>
<tr><td>Applications</td><td>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>
<tr><td>Reagent</td><td>Water bath</td></tr>
<tr><td>Tris0.05M, CsCl 0.26M, $CaCl_2$ 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>
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