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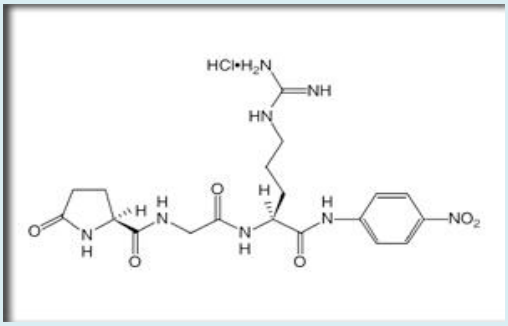
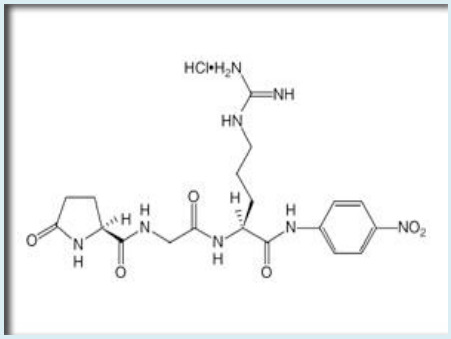
Manufactured By: HYPHEN BioMed

COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF UROKINASE (uPA) CHROMOGENIC SUBSTRATE (HYPHEN BioMed CS-61(44))

	HYPHEN BioMed	**Chromogenix**
Product name	**BIOPHEN CS-61(44)**	**S2444**
Product reference	A229061	82 03 57
Specificity	Recommended substrate for Urokinase	Chromogenic substrate for urokinase
Peptide sequence	PyroGlu—Gly-Arg-pNa-HCl	pyroGlu-Gly-Arg-pNA·HCl
Developed name	L-Pyroglutamyl-glycyl-L-arginine-p-Nitroaniline hydrochloride.	L-Pyroglutamyl-glycyl-L-arginine-p-Nitroaniline hydrochloride.
Chemical structure	 $C_{19}H_{26}N_8O_6$, HCl	
Proposed presentation	25 mg	25 mg
Molarity	~ 54 µmol/vial	-
Bulking agents	Mannitol	Mannitol (40 mg/vial)
Purity grade	> 95%	-
Solubility	>10 mmol/L in H2O	>10 mmol/L in H2O
Molecular weight	462.5 Da (basic structure)	498.9 Da* (*HCl included)
Free pNA content	< 0.05%	NA
E316 nm:	NA	$1.27 .10^4$ mol^{-1} . L . cm^{-1}
Respective reactivities	APC: 1 / FXa: 2 / Plasmin: 1 / Kallicrein: 2 / Thrombin: <1 / Urokinase: 100 Assay conditions must be established for making the substrate totally specific for Urokinase	-
Stability of the lyophilized product	Until the expiration date printed on the vial. (48 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**.	2 mmol/L in H2O is stable for at least 6 months at 2 to 8°C.
Suitable stock solution	According to the research protocol used, the BIOPHEN CS-61(44) 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 H2O.

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<table>
<tr><td>Kinetic data</td><td>Same characteristics</td><td>Urokinase: Ploug U
Km=9 . 10-5 mol/L,
V=3.1. 10-10 mol/min
CTA U Km=6 . 10-5 mol/L,
V=1.3 . 10-10 mol/min
Determined at 37°C in
2.5 mL of 0.05 mol/L Tris
buffer pH 8.8, I 0.05.</td></tr>
<tr><td>Applications</td><td>For in vitro use only.
All research studies and protocols where a source of chromogenic substrate for Urokinase is required.

Suggested protocol:
<table>
<tr><td>Reagent</td><td>Water bath</td></tr>
<tr><td>uPA substrate (at 1mg/ml)</td><td>100 µL</td></tr>
<tr><td>Tris 0.05M , NaCl 0.05M
pH 8.80 buffer</td><td>600 µL</td></tr>
<tr><td colspan="2">Mix and incubate for 2 min at 37°C</td></tr>
<tr><td>uPA at 5000 U/ml</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>400µl</td></tr>
<tr><td colspan="2">Read A405nm against the sample blank.</td></tr>
</table></td><td></td></tr>
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