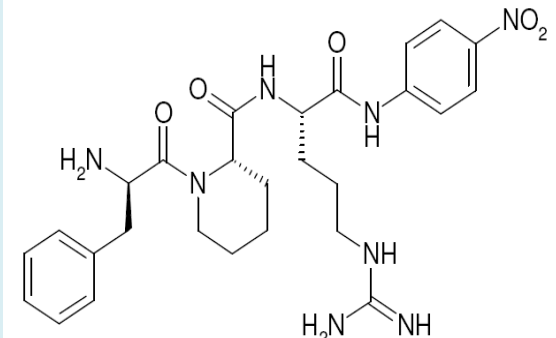
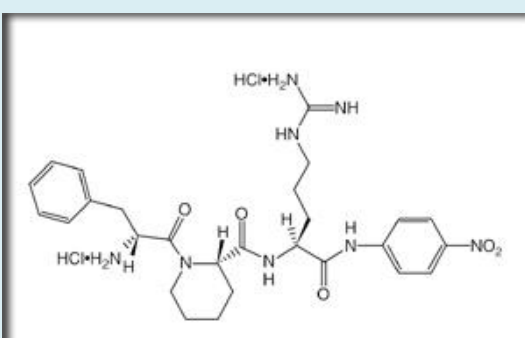


COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF THROMBIN CHROMOGENIC SUBSTRATES (HYPHEN BioMed CS-01(38))

	HYPHEN BioMed	Chromogenix										
Product name	BIOPHEN CS-01(38)	S-2238										
Product reference	A229001 / A229001Z / A229101	82 03 24										
Specificity	Recommended substrate for Thrombin (SIIa-01)	Chromogenic substrate for thrombin										
Peptide sequence	H-D-Phe-Pip-Arg-pNa, 2HCl	H-D-Phe-Pip-Arg-pNA·2HCl										
Developed name	H-D-Phenylalanyl-L-pipecolyl-L-arginine-para-nitroaniline, -dihydrochloride	H-D-Phenylalanyl-L-pipecolyl-L-arginine-p-nitroaniline dihydrochloride.										
Chemical structure	 <p><chem>C27H36N8O5, 2HCl</chem></p>											
Proposed presentation	<ul style="list-style-type: none"> • 25 mg (#A229001) • 100mg (#A229101) • 1g (#A229001Z) 	25 mg										
Molarity	A229001: ~ 45 µmol / vial A229101: ~ 180 µmol / vial A229001Z: ~1810 µmol / vial											
Bulking agents	Mannitol	Mannitol (120 mg/vial)										
Purity grade	> 95%	NA										
Solubility	≥ 5mg/ml in H2O	> 10 mmol/L in H2O										
Molecular Weight	552.6 Da (basic structure)	625.6* (*2HCl 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"> <thead> <tr> <th>Thrombin</th> <th>FXa</th> <th>Plasmin</th> <th>Kallicrein</th> <th>aPC</th> </tr> </thead> <tbody> <tr> <td>100</td> <td>5</td> <td>5</td> <td>60</td> <td>40</td> </tr> </tbody> </table> <p>Assay conditions must be established for making the substrate totally specific for Thrombin.</p>	Thrombin	FXa	Plasmin	Kallicrein	aPC	100	5	5	60	40	
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100	5	5	60	40								
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.										
Stability of the reconstituted product	<ul style="list-style-type: none"> - 7 days at room temperature (18-25 °C) - 3 months at 2-8 °C - Do not freeze. 	1 mmol/L in H2O is stable for more than 6 months at 2-8°C.										



Manufactured By: HYPHEN BioMed

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<p>Suitable stock solution</p>	<p>A229001: According to the research protocol used, the BIOPHEN CS-01(38) chromogenic substrate can be reconstituted 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.</p> <p>A229101: according to the research protocol used (example 20 mL to obtain a substrate concentration of 5 mg/mL)</p> <p>A229001Z: according to the research protocol used.</p>	<p>1-2 mmol/L in H₂O.</p>																
<p>Kinetic data</p>	<p>Same characteristics</p>	<p>Human thrombin: $K_m = 0.7 \cdot 10^{-5}$ mol/L $V = 1.7 \cdot 10^{-7}$ mol/min . NIH-U Bovine thrombin: $K_m = 0.9 \cdot 10^{-5}$ mol/L $V = 2.2 \cdot 10^{-7}$ mol/min . NIH-U Both determined at 37°C in 2.5 mL 0.05 mol/L Tris buffer pH 8.3, I 0.15.</p>																
<p>Applications</p>	<p>For in vitro use only. All research studies and protocols where a source of chromogenic substrate for Thrombin is required.</p> <p>Suggested protocol:</p> <table border="1" data-bbox="400 1137 991 1697"> <tr> <td>Reagent</td> <td>Water bath</td> </tr> <tr> <td>Tris 0.05M, NaCl 0.30M, pH 8.40 buffer</td> <td>400 µL</td> </tr> <tr> <td>Human or Bovine FIIa from 3NIH/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 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>	Reagent	Water bath	Tris 0.05M, NaCl 0.30M, pH 8.40 buffer	400 µL	Human or Bovine FIIa from 3NIH/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 3 min at 37 °C		Citric acid 2%	300µl	Read A405nm against the sample blank.		<p>The substrate has been used for the determination of:</p> <ol style="list-style-type: none"> 1. Prothrombin in plasma 2. Antithrombin in plasma 3. Platelet factor 3 in plasma 4. Heparin in plasma
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<p>Literature reference:</p>	<p>Sim Yee Lean, Paul Ellery, Leesa Ivey, Jim Thom, Robert Oostroyck, Michael Leahy, Ross Baker, Murray Adams, “The effects of tissue factor pathway inhibitor and anti-b-2-glycoprotein-I IgG on thrombin generation”, <i>Haematologica</i> 2006; 91:1360-1366.</p>																	