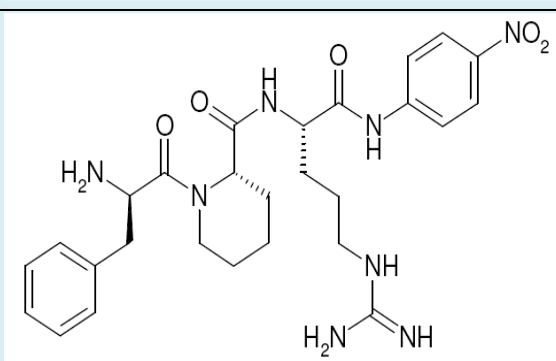
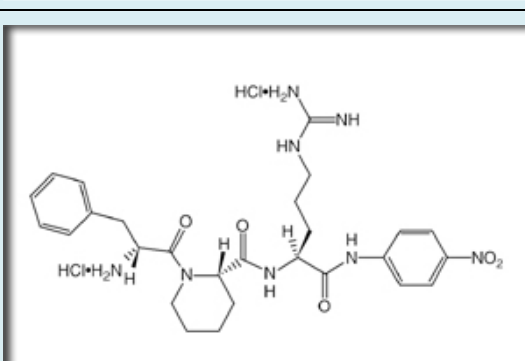


**COMPARISON OF CHARACTERISTICS AND PERFORMANCES OF THROMBIN CHROMOGENIC SUBSTRATES (HYPHEN BioMed CS-01(38))**

	<b>HYPHEN BioMed</b>	<b>Chromogenix</b>										
Product name	<b>BIOPHEN CS-01(38)</b>	<b>S2238</b>										
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</chem>, 2HCl</p>											
Proposed presentation	<ul style="list-style-type: none"> <li>• 25 mg (#A229001)</li> <li>• 10mg (#A229101)</li> <li>• 1g (#A229001Z)</li> </ul>	25 mg										
Molarity	A229001 : ~ 45 $\mu$ M A229101 : ~ 18 $\mu$ M A229001Z : ~1810 $\mu$ M											
Bulking agents	Mannitol	Mannitol (120 mg/vial)										
Purity grade	> 95%	NA										
Solubility	$\geq$ 5mg/ml in H <sub>2</sub> O	> 10 mmol/L in H <sub>2</sub> O										
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	25 °C) - 7 days at room temperature (18-25 °C) - 3 months at 2-8 °C - <b>Do not freeze.</b>	1 mmol/L in H <sub>2</sub> O is stable for more than 6 months at 2-8°C.										

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Suitable stock solution	<p><b>A229001:</b> According to the research protocol used, the BIOPHEN CS-01(38) 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.</p> <p><b>A229101:</b> according to the research protocol used (example 20ml to obtain a substrate concentration of 5mg/ml)</p> <p><b>A229001Z:</b> according to the research protocol used.</p>	1-2 mmol/L in H <sub>2</sub> O.																
Kinetic data	Same characteristics	<p>Human thrombin: <math>K_m = 0.7 \cdot 10^{-5}</math> mol/L <math>V = 1.7 \cdot 10^{-7}</math> mol/min . NIH-U</p> <p>Bovine thrombin: <math>K_m = 0.9 \cdot 10^{-5}</math> mol/L <math>V = 2.2 \cdot 10^{-7}</math> mol/min . NIH-U</p> <p>Both determined at 37°C in 2.5 mL 0.05 mol/L Tris buffer pH 8.3, I 0.15.</p>																
Applications	<p>For in vitro use only.</p> <p>All research studies and protocols where a source of chromogenic substrate for Thrombin is required.</p> <p><b>Suggested protocol:</b></p> <table border="1" data-bbox="400 1093 991 1653"> <thead> <tr> <th>Reagent</th> <th>Water bath</th> </tr> </thead> <tbody> <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> </tbody> </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"> <li>1. Prothrombin in plasma</li> <li>2. Antithrombin in plasma</li> <li>3. Platelet factor 3 in plasma</li> <li>4. Heparin in plasma</li> </ol>
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Literature reference:	<p>Sim Yee Lean, Paul Ellery, Leesa Ivey, Jim Thom, Robert Oostyck, Michael Leahy, Ross Baker, Murray Adams, <b>“The effects of tissue factor pathway inhibitor and anti-b-2-glycoprotein-I IgG on thrombin generation”</b>, <i>Haematologica</i> 2006; 91:1360-1366.</p>																	