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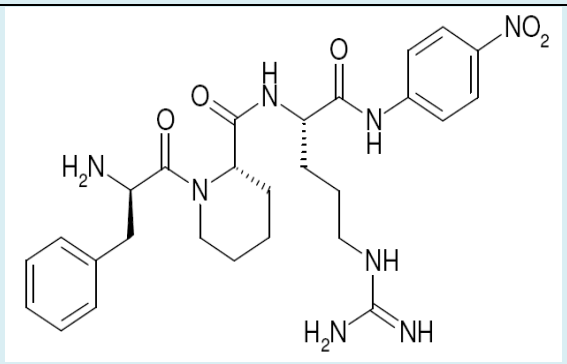
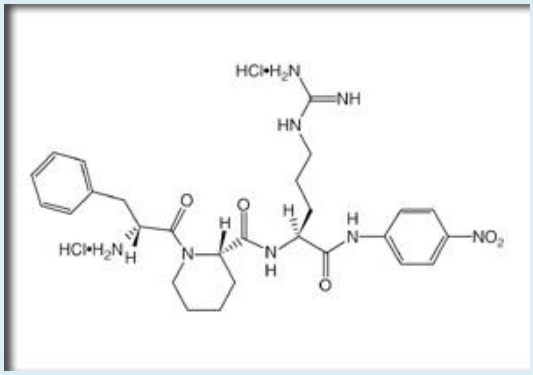
Manufactured By: HYPHEN BioMed

# 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-Larginine- p-nitroaniline dihydrochloride. |
| Chemical structure |  $C_{27}H_{36}N_8O_5$, 2HCl |  |
| Proposed presentation | • 25 mg (#A229001)<br>• 100mg (#A229101)<br>• 1g (#A229001Z) | 25 mg |
| Molarity | A229001: ~ 45 µmol / vial<br>A229101: ~ 180 µmol / vial<br>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 .$10^4$ $mol^{-1}$ . L . $cm^{-1}$ |
| Respective reactivities | Thrombin: 100; FXa: 5; Plasmin: 5; Kallicrein: 60; aPC: 40<br>Assay conditions must be established for making the substrate totally specific for Thrombin. | |
| Stability of the lyophilized product | Until the expiration date printed on the vial.<br>(30 months at 2-8°C from the manufacturing date) | Stable until expiry date if stored at 2-8°C.<br>Avoid exposure to light.<br>The substance is hygroscopic and should be stored dry. |
| Stability of the reconstituted product | - 7 days at room temperature (18-25°C)<br>- 3 months at 2-8 °C<br>-- Do not freeze. | 1 mmol/L in H2O is stable for more than 6 months at 2-8°C. |



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<table>
<tr><td>Suitable stock solution</td><td>A229001: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.<br>A229101: according to the research protocol used (example 20ml to obtain a substrate concentration of 5mg/ml)<br>A229001Z: according to the research protocol used.</td><td>1-2 mmol/L in H2O.</td></tr>
<tr><td>Kinetic data</td><td>Same characteristics</td><td>Human thrombin: Km = 0.7 . 10-5 mol/L V = 1.7 . 10-7 mol/min . NIH-U<br>Bovine thrombin: Km = 0.9 . 10-5 mol/L V = 2.2 . 10-7 mol/min . NIH-U<br>Both determined at 37°C in 2.5 mL 0.05 mol/L Tris buffer pH 8.3, I 0.15.</td></tr>
<tr><td>Applications</td><td>For in vitro use only.<br>All research studies and protocols where a source of chromogenic substrate for Thrombin is required.<br><br>Suggested protocol:
<table>
<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></td><td>The substrate has been used for the determination of:<br>1. Prothrombin in plasma<br>2. Antithrombin in plasma<br>3. Platelet factor 3 in plasma<br>4. Heparin in plasma</td></tr>
<tr><td>Literature reference:</td><td>Sim Yee Lean, Paul Ellery, Leesa Ivey, Jim Thom, Robert Oostryck, Michael Leahy, Ross Baker, Murray Adams, “The effects of tissue factor pathway inhibitor and anti-b-2-glycoprotein-I IgG on thrombin generation”, Haematologica 2006; 91:1360-1366.</td><td></td></tr>
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