

CBS-2238 is the chromogenic substrate D-Phenylalanyl-L-Pipecolyl-p-nitroaniline dihydrochloride that is specific for thrombin and antithrombin. Its amino acid sequence increases its affinity and rates of cleavage > 1000 fold. The method for the determination of activity is based on the difference in absorbance (optical density) between the p-nitroaniline (pNA) formed and the original substrate. The rate of pNA formation, i.e. the increase in absorbance per second at 405 nm, is proportional to the enzymatic activity and its conveniently determined with a spectrophotometer.

Typical properties

<i>Formula</i>	H-D-Phe-Pip-Arg-pNA
<i>Solubility</i>	> 10 mmol.L ⁻¹
<i>Mw</i>	625.6 g.mol ⁻¹
<i>e316 nm</i>	1.27 x 10 ⁴ mol ⁻¹ .L.cm ⁻¹

Standard Buffers & Reagents

- Distilled water
- Tris Buffer: pH 8.4 (6.1 g tris(hydroxymethyl)aminomethane, 10.20 g NaCl, 2.80 g edetate sodium, 10.0 g PEG 6000 and or 2.0 g bovine serum albumin in 1L distilled water)
- Factor IIa solution – reconstitute Factor IIa to 5.0 U.ml⁻¹ in Tris buffer.
- Antithrombin III (AT) solution – reconstitute AT to 0.125 U.ml⁻¹ in Tris buffer.
- CBS-2238 solution – reconstitute to 1.25 mM in Tris buffer.
- Acetic Acid (Stop) solution – 20% (v/v).

Protocol for the determination (factor IIa) activity using a 96 well format

- Add the appropriate concentration of Standard heparin or Test compound in 30 µL of Tris buffer to each well, or 30 mL of Tris buffer as control.
- To each well, add 60 µL of 0.125 U.ml⁻¹ Antithrombin III (AT).
- Gently mix and incubate plate for 2 minutes at 37 °C.
- Add 15 µL of 5.0 U.ml⁻¹ of Factor IIa to each well having Standard heparin or test compound and 15 µL of Tris buffer to control wells.
- Incubate plate for 2 minutes at 37 °C.
- Add 30 µL of CBS-2238 solution to each well and plate for at least 3 minutes at 37 °C.
- Two different measurements can be recorded
 1. End Point measurement:
Add 30 µL -100µL Stopping solution (Acetic acid, 20% (v/v)) after at least 3 minutes incubation at 37 °C and measure the absorbance at 405 nm
 2. Kinetic measurement:
Follow the change in absorbance at 405 nm and calculate the change in absorbance (ΔOD/min)

Packaging, Storage and Stability

Each vial contains 25 mg of CBS-2238 and 120 mg of mannitol as bulking agent. The product is stable until its expiration date if stored, in the dark, at 2-8 °C. Avoid exposure to light. The substance is hygroscopic and should be stored dry. A 1 mmol.L⁻¹ solution of CBS-2238 is stable for than 6 months at 2-8 °C.

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