INterface BIOmaterials BV

CBS-2765 PRODUCT PROFILE

CBS-2765 is the chromogenic substrate N-a-Benzoyloxycarbonyl-D-arginyl-L-Glycyl-L-Arginine-*p*-nitroaniline dihydrochloride that is specific for Factor Xa. 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

Formula	N-a-Z-D-Arg-Gly-Arg-pNA
Solubility	> 40 mmol.L^{-1} in H_20; > 10 mol.l^{-1} in Tris Buffer pH 8.4, I 0.25
Mw	714.6 g.mol^{-1}
e316 nm	1.27 x 10^4 mol^{-1}.L.cm^{-1}

Standard Buffers & Reagents

- Tris Buffer pH 8.4 – dissolve 6.10 g tris(hydroxymethyl)-aminomethane, 2.8 g EDTA, 10.20 g sodium chloride and 1.0 g PEG 6000 in 800 mL of reagent grade water. Adjust pH to 8.4 and dilute to 1L with reagent grade water.
- Factor Xa solution – reconstitute Factor Xa in Tris Buffer to obtain a solution giving an absorbance value between 0.65-1.25 at 405 when assayed as desribed below.
- Antithrombin III (AT) solution – reconstitute AT to 1.0 U.ml^{-1} in Tris buffer.
- CBS-2765 solution – reconstitute to 1 mM in Tris buffer 8.4.
- Acetic Acid (Stop) solution – 20% (v/v)

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

- All reagents and equipment should be at kept 37 ºC.
- 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 25 μL of 1 U.ml^{-1} Antithrombin III (AT).
- Gently mix and incubate plate for 2 minutes at 37 ºC.
- Add 50 μL of Factor Xa to each well having Standard heparin or test compound, and 50 μL of Tris buffer to control wells.
- Incubate plate for 2 minutes at 37 ºC.
- Add 50 μL of CBS-2765 solution to each well and plate for exactly 2 minutes at 37 ºC.
- Add 50 μL of Stop solution to each well and read the absorbance at 405 nm.
- Plot log absorbance vs concentration of Standard (curve 1) and Test compound (curve 2) and divide the slope of curve 2 by curve 1 to obtain the potency value for test compound.

Packaging, Storage and Stability

Each vial contains 25 mg of CBS-2765 and 60 mg of mannitol as bulking agent. The product is stable until its expiration date if stored, in the dark, at 2-8 ºC. Avoid exposure to light. The substance is hygroscopic and should be stored dry. A 1 mmol.L-1 solution of CBS-2765 is stable for than 6 months at 2-8 ºC.

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