N-SUCCINYL-ALA-ALA-PRO-PHE-P-NITROANILIDE

Product Number S 7388
Storage Temperature –0 °C

CAS #: 70967-97-4
Synonyms: Suc-Ala-Ala-Pro-Phe-NA, Suc-AAPF-pNA

Product Description
Appearance: White to faint yellow powder
Molecular Formula: C₃₀H₃₆N₆O₉
Molecular Weight: 624.6
Eₘ (315 nm) = 14,000 M⁻¹ cm⁻¹
Melting point: 184-187 °C

The synthesis of Suc-AAPF-pNA has been reported.¹

Suc-AAPF-pNA is a substrate for alpha-chymotrypsin¹-³ and fungal chymotrypsin-like serine protease⁴ (having a high kcat and a low Km), human leukocyte cathepsin G,⁵,⁶ subtilisin BPN' and its variants,² and protease Q, a recently discovered subtilisin-like protease.⁸ A photometric assay for the determination of chymotrypsin activity in stool samples has been reported.³ It is a substrate for prostate-specific antigen (PA), which exhibits chymotrypsin-like activity.⁹ The substrate is not hydrolyzed by human leukocyte elastase.⁸

Suc-AAPF-pNA was used as the substrate to identify new proteolytic activity in yeast mutants.¹⁰ Peptidyl propyl cis-trans isomerase (PPlase)¹¹-¹⁵ catalyses the cis-trans isomerization of X-Pro peptide bonds. Independently discovered bovine cyclophilin, and the recombinant human form, have been shown to be identical to PPlase.¹⁶ FK-506 binding protein also exhibits PPlase activity.¹⁶ At equilibrium in solution approximately 88% of the substrate peptide has a trans-Ala-Pro bond with the remaining in the cis form.¹⁴-¹⁷ The trans form of the peptide is readily cleaved by chymotrypsin. The remaining cis form can be enzymatically converted to the trans form by PPlase.¹⁶,¹⁷

Reconstitution of the dried substrate in LiCl/tetrahydrofuran shifts the cis-trans equilibrium to 5-40% cis-Ala-Pro isomer. In this solvent, the substrate has been used in an improved coupled assay for PPlase activity determination.¹⁶

Enzymatic cleavage of 4-nitroanilide substrates yields 4-nitroaniline (yellow color under alkaline conditions). Sigma uses the molar extinction coefficient (Eₘ) of 8,800 M⁻¹ cm⁻¹ at 410 nm, pH 7.5, when using 4-nitroanilide substrates in enzyme assays. Other reaction conditions and molar extinction coefficients have been reported.³,¹⁰,¹⁸

Storage/Stability
Suc-AAPF-pNA has a shelf life of 3 years when stored desiccated at –0 °C.

Preparation Instructions
Suc-AAPF-pNA is soluble in N,N-dimethylformamide (DMF) at 25 mg/ml producing a clear, light yellow solution. It is also soluble in 5% dimethylformamide (v/v) in buffer² and in distilled water at 4 mg/ml. A 20 mM solution in dimethyl sulfoxide (DMSO) has been prepared and stored as a stock solution at 4 °C.⁶ Stock solutions in DMSO were diluted in 0.10 M HEPES buffer, pH 7.5, for the assay of cathepsin G.⁶

Suc-AAPF-pNA is soluble at 10 mM in 0.2 M Tris-HCl buffer, pH 8.0. In this buffer, a 1 mM solution of the substrate spontaneously hydrolyzes at a rate of about 0.1% per day at 4 °C.¹

It is recommended to prepare aqueous solutions of Suc-AAPF-pNA immediately prior to use. Aqueous solutions of the substrate were kept on ice during experiments.²

References
4. Larcher, G., et al., Purification and characterization of a fibrinogenolytic serine proteinase from