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ProductInformation

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: $C_{30}H_{36}N_6O_9$
Molecular Weight: 624.6
E_M (315 nm)[1,2] = 14,000 M^{-1} cm^{-1}
Melting point:[1] 184-187 °C

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

Suc-AAPF-pNA is a substrate for alpha-chymotrypsin[1-3] and fungal chymotrypsin-like serine protease[4] (having a high k_{cat} and a low K_m), human leukocyte cathepsin G,[5,6] subtilisin BPN' and its variants,[7] 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.[5]

Suc-AAPF-pNA was used as the substrate to identify new proteolytic activity in yeast mutants.[10] Peptidyl propyl *cis-trans* isomerase (PPIase)[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 PPIase.[16] FK-506 binding protein also exhibits PPIase activity.[16,17] 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 PPIase.[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 PPIase activity determination.[16]

Enzymatic cleavage of 4-nitroanilide substrates yields 4-nitroaniline (yellow color under alkaline conditions). Sigma uses the molar extinction coefficient (E_M) of 8,800 $M^{-1}cm^{-1}$ at 410 nm, pH 7.5, when using 4-nitroanilide substrates in enzyme assays.[6] 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 buffer[10] and in distilled water at 4 mg/ml.[2]

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

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