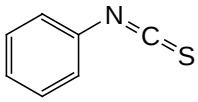
INSTRUCTIONS

PITC (Edman's Reagent)

26922

0863.2

Number	Description
26922	**PITC (Edman's Reagent),** 10 × 1mL Molecular Weight: 135.19 Formula: C_7H_5NS



Storage: Upon receipt store at room temperature.

Introduction

Thermo Scientific PITC (phenylisothiocyanate), also known as Edman's Reagent, enables the sequential degradation of amino acids in a polypeptide chain, yielding primary structural information.[1,2] PITC reacts readily with amino acids at alkaline pH. Precolumn derivatization results in phenylthiocarbamyl derivatives (PTC-amino acids) that can be separated and quantified using reverse-phase HPLC.[3-6] This method produces stable products with all amino acids, including proline. PITC is volatile, making it possible to remove excess reagent *in vacuo*, thereby minimizing the possibility of reagent interference. Detection of picomole quantities of the derivatives can be achieved using a UV detector at 254nm. PITC derivatization followed by reverse-phase chromatography can be used for identification and quantitation of methylated, halogenated, phosphorylated and sulfonated amino acids.[6]

Unlike Fmoc-chloride, PITC does not yield disubstituted tyrosine or histidine derivatives. PTC-amino acids demonstrate improved stability at pH 5-7.5 as well as increased stability at room temperature over *o*-phthalaldehyde (OPA)-amino acid adducts. Also, unlike OPA, PITC enables the direct analysis of secondary amino acids.

Example Protocol for Derivatizing Amino Acid Standard H

A. Additional Materials Required

- Amino Acid Standard H (Product No. 20088, 10 × 1mL, or Product No. 20089, 10mL)
- Coupling Solution: acetonitrile:pyridine:triethylamine:water (10:5:2:3)
- Analysis Solvent: 0.05M ammonium acetate or water:acetonitrile (7:2)

B. Method

1. Dry 10µL of Amino Acid Standard H in a small test tube. Dissolve dried standard in 100µL Coupling Solution.

   Note: Make sure that all of the HCl is evaporated before derivatization.
2. Dry standard solution by rotary evaporation. Dissolve the residual amino acids again in 100µL Coupling Solution.
3. Add 5µL of PITC and allow reaction to proceed for 5 minutes at room temperature.
4. Evaporate sample to dryness by rotary evaporation under high vacuum.
5. Dissolve the resulting PTC-amino acids in 250µL of Analysis Solvent.
6. Analyze 1-10µL (100 to 1000pmol of each amino acid) by reverse-phase HPLC with UV detection at 254nm.

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Related Thermo Scientific Products

25104	**Pyridine,** 100g
51101	**Acetonitrile,** 1L
28901	**Trifluoroacetic Acid,** 500mL
25003	**Heptafluorobutyric Acid,** 100mL

Cited References

1. Edman, P. (1950). Preparation of phenylthiohydrantoins from natural amino acids. *Acta Chem Scand* **4:**277-82.
2. Edman, P. and Begg, G. (1967). A protein seqenator. *Eur J Biochem* **1(1):**80-91.
3. Heinrikson, R.L. and Meridith, S.C. (1984). Amino acid analysis by reverse-phase high-performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. *Anal Biochem* **136:**65-74.
4. Schoze, H. (1985). Determination of phenylthiocarbamyl amino acids by reverse-phase high-performance liquid chromatography. *J Chromatogr* **350:**453-60.
5. Ebert, R.F. (1986). Amino acid analysis by HPLC: Optimized conditions for chromatography of phenylthiocarbamyl derivatives. *Anal Biochem* **154:**431-5.
6. Cohen, S.A. and Strydom, D.J. (1988). Amino acid analysis utilizing phenyliosthiocyanate derivatives. *Anal Biochem* **174:**1-16.

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