INSTRUCTIONS

Phenylisothiocyanate (PITC)

TS-26922 0863w

Product Description

Number	Description
TS-26922	**Phenylisothiocyanate (PITC, Edman's Reagent),** ***10 x 1 ml***
	Store at room temperature.

Introduction

PITC, 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 in 5-10 minutes at room temperature (RT). Precolumn derivatization results in phenylthiocarbamyl derivatives (PTC-amino acids) that can be separated and quantified in 30 minutes using reverse-phase HPLC.[3-6] This method produces stable products with all amino acids, including proline. The PITC reagent 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 254 nm. PITC has only been applied for precolumn derivatizations due to the required lengthy sample preparation and derivatization steps. PITC derivatization, followed by reverse phase chromatography, can be used in the 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 from pH pH 5-7.5 as well as increased stability at RT over *o*-Phthalaldehyde (OPA)-amino acid adducts. Also, unlike OPA, PITC permits the direct analysis of secondary amino acids.

Example Protocol: Derivatization of Pierce Amino Acid Standard H with PITC.[3]

Materials Required

Amino Acid Standard H (Prod. No. 20088, *10 x 1 ml*, or Prod. No. 20089, *10 ml*)

Coupling Solution: Acetonitrile: Pyridine: Triethylamine: H_2O (10:5:2:3)

PITC (Phenylisothiocyanate)

Analysis Solvent: 0.05 M ammonium acetate, water or water: acetonitrile (7:2).

Method

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

   Note: Make certain that all of the HCl is evaporated before derivatization.

2. Dry standard solution by rotary evaporation. Dissolve the residual amino acids again in 100 µl Coupling Solution.

3. Add 5 µl of PITC.

4. Allow reaction to proceed for 5 minute at RT.

5. Evaporate sample to dryness by rotary evaporation under high vacuum.

6. Dissolve the resulting PTC-amino acids in 250 µl of Analysis Solvent.

7. Analyze quantities of 1 to 10 µl (100 to 1,000 picomoles of each amino acid) by reverse-phase HPLC with UV detection at 254 nm.

References

1. Edman, P. (1950). Preparation of phenylthiohydrantoins from natural amino acids. *Acta. Chem. Scand*. **4**, 277-282.

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.

Schoze, H. (1985). Determination of phenylthiocarbamyl amino acids by reverse-phase high-performance liquid chromatography. *J. Chromatogr*. **350**, 453-460.

4. Ebert, R.F. (1986). Amino acid analysis by HPLC: Optimized conditions for chromatography of phenylthiocarbamyl derivatives. *Anal. Biochem*. **154**, 431-435.

Cohen, S.A. and Strydom, D.J. (1988). Amino acid analysis utilizing phenyliosthiocyanate derivatives. *Anal. Biochem.* **174**, 1-16.

5

Current versions of product instructions are available at *www.thermo.com/columns*.