INSTRUCTIONS

Vacuum Hydrolysis Tubes

29570 29571 29572

2298.0

Number	Description
29570	**Vacuum Hydrolysis Tube, 1mL, 8mm × 60mm**
29571	**Vacuum Hydrolysis Tube, 6mL, 10mm × 150mm**
29572	**Vacuum Hydrolysis Tube, 18mL, 19mm × 100mm**

Introduction

The Thermo Scientific Vacuum Hydrolysis Tubes are precision-designed for use in protein hydrolysis and subsequent analysis of protein and peptide amino acid compositions. The heavy-walled borosilicate glass construction ensures complete compatibility with all commonly used acid hydrolysis reagents. Acid hydrolysis at elevated temperatures, in vacuo, will hydrolyze most proteins and peptides to their amino acid components without significant decomposition.

Vacuum hydrolysis methods commonly use 6N constant boiling hydrochloric acid (HCl), a mixture of HCl and propionic acid, 3N mercaptoethanesulfonic acid or 4N methanesulfonic acid. The constant boiling HCl method uses standard protein hydrolysis at 105-110°C for 16-24 hours.[1] Using the HCl/propionic acid method, hydrolysis times can be shortened to 15 minutes at 160°C.[2] Tryptophan is easily degraded by acid hydrolysis in 6N HCl. The presence of oxygen, heavy metals and carbohydrates are contributing factors to this degradation. A 22-hour hydrolysis method was developed to improve tryptophan recovery from protein hydrolysis samples by using 3N mercaptoethanesulfonic acid.[3,4] A second method to improve tryptophan recovery involves hydrolysis at 115°C using 4N methanesulfonic acid.[5,6]

Although originally designed for protein hydrolysis, Vacuum Hydrolysis Tubes are excellent vessels for conducting other chemistries that require in-vacuo conditions (e.g., hydrazinolysis), sample concentration or lyophilization techniques.

Important Product Information

- Wear hand and eye protection when handling glass equipment. The heavy-walled construction will help prevent accidental breakage; however, avoid exerting unnecessary twisting forces upon the unit when applying tubing. Before each use, carefully inspect tubes for small cracks in the glass. Do not use the tube if there are cracks in any area of the glass; to do so will risk sudden failure (implosion).
- Vacuum Hydrolysis Tubes are not intended for atmospheric hydrolysis. Do not use these tubes in situations in which internal pressures can build.
- Avoid leaving any sample residue in the upper portion of the tube; particularly at the threads; the top of the reservoir; the stricture and the plug seals.
- Use sample volumes that are ≤ 1/3 of the stated reservoir volume. This will help prevent sample loss during the vacuum application, when foaming may occur.
- Attaching vacuum tubing from the side arm of the tube to a three-way stopcock that is connected to a vacuum source and a supply of purified nitrogen or argon will allow you to alternate between vacuum and an inert gas, a desirable technique to ensure the complete removal of oxygen from the sample.
- Do not heat tubes to greater than 110°C in an oven, because plug components are not rated to withstand these temperatures. The tubes can withstand temperatures up to 200°C for 48 hours in Thermo Scientific Reacti-Therm Heating Modules.

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Standard Procedure for Sample Vacuum Hydrolysis

1. Unscrew the plug from the unit. Use a small-diameter glass pipette (disposable Pasteur) or syringe to introduce the sample into the bottom reservoir of the tube.
2. Insert plug and screw it down just enough to leave a small passageway between the plug and the glass at the stricture point. Secure tube and attach the vacuum source to the side arm.
3. Optional: To prevent excessive foaming and bumping during the evacuation process, samples may be frozen before applying the vacuum.
4. After the tube has been evacuated, seal unit by slowly screwing the plug until it is flush with the glass surface at the stricture. Do not over-tighten, which may cause tube to break, the black cap to crack or the plug to be damaged.
5. Apply heat to samples. After vacuum hydrolysis, allow samples to cool to room temperature.
6. Close the vacuum source and relieve the vacuum by carefully unscrewing the plug and allowing air or, preferably, an inert gas to slowly enter the tube. A three-way stopcock will simplify this procedure.
7. With the tube securely fastened, the sample may be concentrated via vacuum, with or without heating of the tube, as dictated by your protocol. Alternatively, the hydrolysis reagent may be removed by lyophilization (connect the tube directly to the lyophilizer) or via a stream of nitrogen/argon directed at the surface of the sample.
8. Most amino acid analysis protocols require the concentrated sample to be reconstituted in a sample dilution buffer. Sample dilution buffer may be added to the tube by removing the plug and using a pipette to add buffer.
9. Remove sample using a small diameter pipette.
10. Thoroughly clean tube as soon as possible after use. Thermo Scientific PCC-54 Detergent (Product. No. 72288) will remove protein residues and heavy metals. After cleaning, rinse tube with distilled water and HCl and oven-dry at ≤ 110°C. Avoid inserting brushes through the top of the unit, because it is possible to scratch the inner glass surfaces with metal stems on brushes. Scratches may cause difficulty in resealing the tube and result in unit failure.

Related Thermo Scientific Products

72288	**PCC-54 Detergent Concentrate,** 3L
28904	**Trifluoroacetic Acid (TFA), Sequencing grade,** 10 × 1mL ampules
28905	**Formic acid, 99+%,** 10 × 1mL ampules

General References

Eveleigh, J.W., and Winter, G.D. (1970). Protein Sequence Determination, Ed. Needleman, S. B., Springer-Verlag, pp 92-5.

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Penke, B., *et al.* (1974). New acid hydrolysis method for determining tryptophan in peptides and proteins. *Anal Biochem* **60:**45-50.

Creamer, L.K. and Matheson, A.R. N.Z.J. (1976). The use of mercaptoethanesulfonic acid as a hydrolyzing agent for the determination of tryptophan in proteins. *Dairy Sci Technol* **11:**211-2.

Liu, T.-Y. and Chang, Y.H. (1971). Hydrolysis of proteins with p-toluenesulfonic acid. *J Biol Chem* **246:**2842-8.

Simpson, R.J., *et al.* (1976). Complete amino acid analysis of proteins from a single hydrolysate. *J Biol Chem* **251:**1936-40.

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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