

Tertbutyldimethylsilyl (TBDMS) derivatization of amino acids

1. Introduction

This protocol describes the derivatization of biomass amino acids for subsequent GC/MS analysis.

2. Required Materials and Equipment

- Biomass sample, 1 mL ($OD_{600} \sim 1-2$)
- 6 N Hydrochloric Acid, HCl
- Pyridine
- MTBSTFA + 1% TBDMCS, 1mL ampoules (Pierce Biotechnology, Cat# 48927)
- 1.5 mL Eppendorf Tubes
- GC Injection Vials
- Evaporator
- Heating block

3. Biomass hydrolysis and sample preparation

- Centrifuge biomass sample for 5 min at 14,000 rpm
- Remove supernatant
- Add 500 μ L of 6 N HCl to biomass pellet
- Vortex vigorously for 10 sec
- Heat for 18-24 hr at 110°C on heating block
- Centrifuge for 5 min at 14,000 rpm to remove cell debris
- Transfer supernatant into new Eppendorf tube
- Evaporate to dryness under air flow at 65°C (in fume hood)

4. Derivatization protocol

- Dissolve dried sample in 50 μ L of pyridine (in fume hood)
- Add 50 μ L of MTBSTFA + 1% TBDMCS (in fume hood)
- Incubate for 30 min at 60°C on heating block
- Centrifuge for 5 min at 14,000 rpm to remove debris/precipitation
- Transfer supernatant to GC injection vial
- Label vial with: your initials, date, and sample number
- Store samples at 4°C if not used immediately