

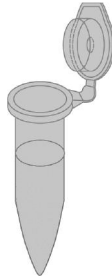
Instructions for Sample Preparation: Ethanol Procedure

Pure cultures are essential for microbial identification. Ethanol sample preparation procedures can be used for submitting bacterial samples for 16S Sequencing and AccuPRO-ID® Microbial Identification, or submitting yeast and filamentous fungi samples for ITS2 Sequencing.

Bacteria & Yeast Samples:

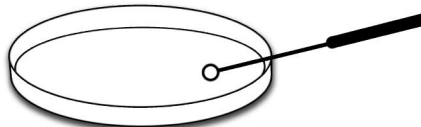
1. Complete the Identification Request Form (IRF)

- On each microcentrifuge tube, attach a label with the Sample ID number corresponding to the IRF.



2. Prepare tube and harvest sample

- Add 300µL of sterile, nuclease-free water to each tube.
- Using a sterile inoculation loop, harvest about 3 large colonies for a generous loopful of sample. Evenly suspend the organism in the water by vortexing or mixing well. After suspending in water, the solution should have the turbidity equivalent to the 4-8 McFarland Standards. Avoid depositing agar into the suspension.



Note: When submitting samples for AccuPRO-ID microbial identification, always use cultures that are 24-48 hours old. For slow growing organisms, harvest as soon as there is visible growth. Do not refrigerate plates prior to preparation.

Note: If the organism is very mucoid, has a chunky uneven appearance, or fails to suspend homogeneously, an Eppendorf pestle should be used to assist in evenly suspending the organism in water.

Note: If limited biomass is available, suspend as much organism as possible in 100µL of sterile, nuclease-free water and add 300µL of absolute ethanol.

3. Add ethanol

- Add 900µL of absolute ethanol to each suspension, secure the cap, and gently mix. After adding ethanol, the solution should have a turbidity equivalent to the 1-2 McFarland Standards.

4. Ship to Accugenix with IRF

- Parafilm tubes to prevent caps from opening during transit, then place in a ziplock bag and shipping box. Add foam or bubblewrap to prevent shifting during transport.

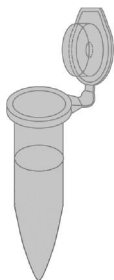


- Refer to the Accugenix Shipping Guide for specific labeling and documentation required before shipping.

Filamentous fungi samples:

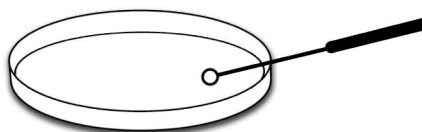
1. Complete the Identification Request Form (IRF)

- On each microcentrifuge tube, attach a label with the Sample ID number corresponding to the IRF.



2. Prepare tube and harvest sample

- Add 300µL of sterile, nuclease-free water to each tube.
- Using a sterile inoculation loop, harvest 4 or 5 pieces of mold, each approximately 3.0-5.0mm in diameter from the grown medium. Avoid obtaining too much agar. If possible, obtain samples from the edges of mold colonies. *Avoid using overgrown mold cultures.*



- Grind the mold as much as possible in the tube using an Eppendorf pestle to assist in evenly suspending the organism in the water.



Note: If limited biomass is available, suspend as much organism as possible in 100µL of sterile, nuclease-free water and add 300µL of absolute ethanol.

3. Add ethanol

- Add 900µL of absolute ethanol to each suspension, secure the cap, and gently mix.

4. Ship to Accugenix with IRF

- Ensure tubes are securely closed.
- Refer to the Accugenix Shipping Guide for specific labeling and documentation required before shipping.

Material Recommendations

Item	Supplier	Product #
1.5mL Eppendorf Safe-Lock microcentrifuge tube (500 Pack)	Eppendorf	022363212
Absolute Ethanol (North America)	Sigma Aldrich	E7023
Absolute Ethanol (International clients)	Sigma Aldrich	24102
Eppendorf Micropestle 022365622	Eppendorf	
Sterile, Nuclease-free water	Various	
4-8 McFarland Standard	Various	
1-2 McFarland Standard	Various	

Contact your local laboratory supply representative for assistance in obtaining needed materials.

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