charles river

MICROBIAL SOLUTIONS

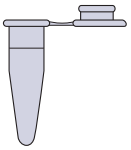
Instructions for Sample Preparation:

Liquid Ethanol Procedure

Pure cultures are essential for microbial identification. This procedure can be used for submitting bacterial and yeast samples for AccuPRO-ID®, AccuGENX-ID® (BacSeq and FunITS), Accugenix-ST®, and for bacterial samples for Ribotyping (BacRib).

1. Complete the Identification Request Form (IRF).

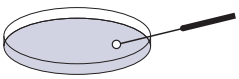
On each microcentrifuge tube, attach a label on the lid or near the top of the tube with the sample ID number corresponding to the IRF.



2. Prepare tube and harvest sample.

Bacteria and Yeast Samples

- Add 300 µL 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 with a pipette. After suspending in water, the solution should have a turbidity equivalent to 4-8 McFarland standard. Avoid depositing agar into the suspension.



Note: When submitting samples for AccuPRO-ID® yeast or bacterial 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 homogenously, 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 100 µL of sterile, nuclease-free water and add only 300 µL of absolute ethanol.

Filamentous Fungi Samples

- Add 300 µL of sterile, nuclease-free water to each tube.
- Using a sterile inoculation loop, harvest 4 or 5 pieces of mold, each approximately 3.0-5.0 mm in diameter, from the grown media. If possible, obtain samples from the edges of mold colonies and avoid obtaining too much agar. Avoid using overgrown mold cultures.
- Grind the mold as much as possible in the tube using an Eppendorf® micropestle to assist in evenly suspending the organism in the water.

3. Add ethanol.

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

4. Ship to Charles River with IRF.

- Apply Parafilm® to tubes to prevent the caps from opening during transit. To keep the tubes from being crushed during transport, place padding around the samples. Place the samples into a plastic zip bag, then insert the IRF and bagged samples into an air cushioned envelope for transport.
- Refer to the Charles River Shipping Guide for specific labeling and documentation required before shipping.

Identification Request Forms and additional information about our services and capabilities can be found online at www.criver.com/accugenix.

EVERY STEP OF THE WAY

Material Recommendations

Item	Supplier	Product #
1.5 mL Eppendorf® Safe Lock Tubes™ *(500 Pack)*	Eppendorf®	022363212
Absolute Ethanol *(North America)*	Sigma-Aldrich®	E7023
Absolute Ethanol *(International)*	Sigma-Aldrich®	24102
Eppendorf® Micropestle	Eppendorf®	022365622
Sterile, Nuclease-Free Water	Various	
4-8 McFarland Standard	Various	
1-2 McFarland Standard	Various	
1 μL Sterile Inoculation Loop	Various	

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

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