

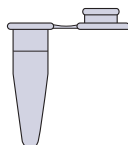


## Instructions for Sample Preparation: Dried Ethanol Procedure

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).

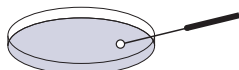
On each microcentrifuge tube, attach a label on the lid or near the top of the tube with the sample ID number corresponding to the IRF.



### 2. Prepare tube and harvest sample.

#### Bacteria and Yeast Samples

- Add 300  $\mu\text{L}$  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 homogeneously, an Eppendorf® pestle should be used to assist in evenly suspending the organism in water.

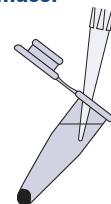
**Note:** If limited biomass is available, suspend as much organism as possible in 100  $\mu\text{L}$  of sterile, nuclease-free water and add only 300  $\mu\text{L}$  of absolute ethanol.

#### Filamentous Fungi Samples

- Add 300  $\mu\text{L}$  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 and concentrate the cell mass.

- Add 900  $\mu\text{L}$  of absolute ethanol to each suspension, secure the cap and gently mix. The solution should have a turbidity equivalent to 1-2 McFarland standard.
- Place the sample in a microcentrifuge\* and spin.
- After centrifugation, ensure that a pellet has formed. Slowly decant the sample by pouring or pipetting off the supernatant, taking care not to disturb or discard the pellet.
- Place the sample in a microcentrifuge and spin again.
- Pipette off any residual supernatant.
- Leave the sample open at room temperature until all residual supernatant has evaporated.



\* See Material Recommendations for microcentrifuge specifications.

#### 4. Ship to Charles River with IRF.

- Close the cap to all samples, then 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](http://www.criver.com/accugenix).

#### 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.

#### Recommended Microcentrifuges and Settings

<b>Minifuge</b> (8 tubes) VWR Cat. #9300-196	6,000 rpm max for 6 minutes
<b>Microfuge</b> (12 tubes) VWR Cat. #37001-294	10,000 rpm for 3 minutes or 5,000 rpm for 5 minutes
<b>Large Microfuge</b> (24 tubes) VWR Cat. #37001-300	13,000 rpm max for 2 minutes

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