

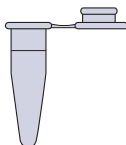


Instructions for Sample Preparation: Alkaline Lysis Extraction

Pure cultures are essential for microbial identification. This procedure can be used for submitting bacterial isolates for Accugenix® 16S sequencing and/or MLST/SLST, or submitting yeast and filamentous fungi samples for ITS2 sequencing.

1. Complete the Identification Request Form (IRF).

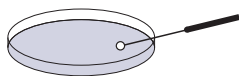
On each microcentrifuge tube, attach a label on the lid or near the top of the tube with the sample ID number corresponding to the IRF.



2. Prepare tube and harvest sample.

Bacteria and Yeast Samples

- Add 100 μL of the alkaline lysis reagent to each of the microcentrifuge tubes.
- Using a sterile 1 μL size inoculation loop, harvest a loopful of one pure colony. Avoid depositing agar into the suspension. Mix well by twisting the loop back and forth until the entire sample is deposited into the liquid and no longer on the loop.



- Evenly suspend the organism in the solution by vortexing or mixing well with a sterile pipette tip.
- The solution should have the turbidity equivalent to 0.5–2 McFarland standard.

Filamentous Fungi Samples

- Add 100 μL of the alkaline lysis reagent to each of the microcentrifuge tubes.
 - Using a sterile 1 μL size inoculation loop, harvest a loopful of mold approximately 3.0–5.0 mm in diameter from the growth medium. Avoid obtaining too much agar. If possible, obtain the sample from the edges of the mold colony and try to avoid spores.
- Note:** Ideally collect the sample from a younger mold because they typically have less spore formations.
- Grind the mold as much as possible in the tube using an Eppendorf® or Kontes™ pestle to assist in evenly suspending the organism in the solution.

3. 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.

Alkaline Lysis Reagent Preparation

(25 mM NaOH and 0.2 mM EDTA solution)

Once prepared, the alkaline lysis reagent is good for 1 year if stored at room temperature.

- For a 25 mM NaOH, add 1.0 gram of pellets to a clean HDPE or LDPE bottle containing 1,000 mL of LC-grade water.
- Add 400 μ L of 0.5 M EDTA solution.
- Check the pH and adjust to a pH level of 13 if necessary by adding additional NaOH.

Material Recommendations

Item	Supplier	Product #
NaOH pellets	Various	
0.5 M EDTA Solution	Various	
LC-Grade Water	Various	
1.5 mL Eppendorf® Safe Lock Tubes™	Eppendorf®	022363212
0.5-2 McFarland standard	Various	
1 μ L Sterile Inoculation Loop	Various	
Eppendorf® Micropestle	Eppendorf®	022365622
Kontes™ Pellet Pestle™	Fischer	749521-1590
pH Indicator Strips (0-14)	Various	

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

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