Rapid Prep for PCR Applications Protocol 2

Lyse & Purify (96 Wells)
Cell Suspensions and Cell Pellets

Rapidly lyse and purify DNA from cell suspensions or cell pellets. This kit contains materials for processing 96 samples.

Additional Items Needed: (Not included; see Suggested Supplier list below)

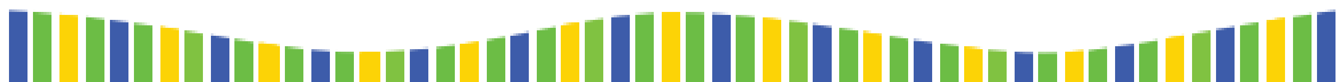
1. Additional Items **Required** for Lyse and Purify
   a. One (1) sterile 96-well PCR plate
   b. Plate sealing film
   c. Sterile Laboratory Grade Water
   d. Fresh 70% ethanol
   e. TE Buffer - 10mM Tris, 1mM EDTA, pH 8.0
   f. 0.4M KOH Solution
   g. Magnetic Rack ('bar' or 'post' suggested for higher DNA yield)
2. Suggested items for rapid processing
   a. Repeater Pipette with multi-volume tips
   b. Single Channel Manual Pipettes
   c. Multichannel Pipette 0.5-10 µL working volume
   d. Centrifuge with 96-well plate adaptor

Kit Contents for Shoreline Biome Lyse and Purify

1. Lysis-1 (96-well PCR plate, dried solution in each well)
2. Purification Buffer (two 8 mL bottles with **white** caps)

Shoreline Biome Lyse and Purify Protocol

1. **Make 0.4M KOH**. Measure 0.135g KOH into 15 mL tube, then add 6 mL H_2O. 0.4M KOH expires after 7 days.
2. **Make 70% Ethanol**. Add 17.5 mL 100% ethanol to 7.5 mL sterile laboratory grade water
3. Bring Purification Buffer (**white** capped bottle) to room temperature.
4. Remove film covering the 96-well PCR plate containing dried Lysis-1.
5. **Sample Type:**
   5.1. **Cell suspension:**
      5.1.1. **Transfer 50 µL of cell suspension** (between 10^6 and 10^8 cells) to Lysis-1 plate. Do not mix.
   5.2. **Cell pellets:**
      5.2.1. Reconstitute the cell pellet by adding 50 µL Molecular Biology Grade water, TE, or PBS and pipetting up and down to suspend cells.
      5.2.2. **Transfer 50 µL** of the cell suspension (between 10^6 and 10^8 cells) to Lysis-1 plate. Do not mix.
6. **Add 50 µL 0.4M KOH** to each well position in the 96-well plate. Do not mix. Use of a repeater or multichannel pipette is recommended to save time. A precipitate will form in the wells.
7. **Cover the sample plate with PCR film**, load the sample plate into the PCR machine, close and lock the lid, and **heat samples to 95°C for 5 minutes**. Precipitate will dissolve.
8. **Place sample plate on ice for 2 minutes.** Precipitate will re-form.
9. Vortex Purification Buffer bottles (**white** cap) to re-suspend any brown magnetic beads that may have settled.



10. Remove sample plate from ice and carefully remove PCR film.
11. Add **100 µL of Purification Buffer** to each well of the 96-well sample plate and **pipette to mix**.
12. **Cover the sample plate with a *new* PCR film**, load sample plate onto the PCR machine, close and lock the lid, and **incubate solution for 3 minutes at 50˚C** to allow DNA to bind to beads
13. After incubation, place plate on magnetic rack to pellet beads with bound DNA (~60 seconds), carefully remove PCR film.
14. Once all beads are pelleted, **remove all supernatant SLOWLY and discard**. Avoid aspirating beads.
15. **Remove plate from magnet, add 100 µL 70% ethanol** (at room temperature) to each well, and re-suspend beads by pipetting gently up and down.
16. **Return plate to magnet and allow beads to pellet** (~30 seconds)
17. **Pipette off residual ethanol and let air dry for 2 - 3 minutes**. Over-drying will make eluting DNA more difficult, so watch to ensure ethanol is mostly gone but bead pellet does not begin to shrink and crack.
18. **Remove plate from magnet.**
19. Add **20 µL of 1X TE buffer** to each well and briefly pipette up and down to re-suspend beads. Solution should turn brown in color to reflect beads mixed homogeneously throughout.
20. **Incubate plate at room temperature for 2 minutes.**
21. Place plate on magnetic rack for 30 - 60 seconds to **pellet magnetic beads. DNA is now in solution.**
22. **Transfer supernatant containing eluted DNA** into a **clean** PCR plate on ice, avoiding pelleted beads.
23. **Remove plate with pelleted beads from rack**.
24. **Add another 20 µL 1X TE buffer** onto bead pellets, gently pipette up and down to suspend beads.
25. **Incubate plate for 2 minutes at room temperature**.
26. Return to magnetic rack. Allow beads to pellet to sides for 30 - 60 seconds.
27. **Remove supernatant containing DNA** and combine with first eluate in clean plate, avoiding pelleted beads.
28. **Store eluted DNA at +4°C**.

NOTES: DNA is **denatured**, due to KOH treatment during lysis, which is optimal for PCR. Store Lysis Mix at +4°C for up to 10 days, or frozen at -20°C for 3 months. Avoid repeated freeze/thaws.

Suggested Suppliers

Dot Scientific Inc.: 96-well plate, #353-PCR or #354-PCR
Fisher Scientific: Biotech Grade Water BP24854; Potassium Hydroxide P250; TE BP24731
Thermo Fisher: Invitrogen Dyna Mag 96 side magnetic rack, #12331D or Magnetic stand-96 #AM10027

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