DNA purification protocol using Epicentre MasterPure™ Complete DNA and RNA Purification Kit

OMNIgene® family of swab-based kits (OMR-110, OMR-120, OMR-130)

This laboratory protocol is used for the preparation of a sample collected and stabilized in any of the OMNIgene family of swab-based kits (OMR-110, OMR-120, OMR-130) for subsequent extraction of microbial DNA using the Epicentre MasterPure™ Complete DNA and RNA Purification Kit.

Required reagents

- Proteinase K (PK), 80 mg/mL
- Ready-Lyse™ Lysozyme Solution (Epicentre, Cat. No. R1802M)
- Epicentre MasterPure™ Complete DNA and RNA Purification Kit (Cat. No. MC85200)
  – Refer to the manual in the kit for a detailed extraction protocol†

Equipment required

- Equipment listed as referenced in the Epicentre MasterPure™ Complete DNA and RNA Purification Kit (Cat. No. MC85200)†

Procedure

<table>
<thead>
<tr>
<th>Sample prep steps</th>
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<tbody>
<tr>
<td>1. Add 5 µL of PK* (80 mg/mL) to the 1 mL sample collection tube and vortex. Incubate for 1 hour at 50°C water bath or 2 hours in a dry 50°C incubator. Ensure that the swab is in contact with the chemistry.</td>
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<td>*Recommended PK: Epicentre PK (Cat. No. MPRK092) or QIAGEN® Protease (Cat. No. 19155)</td>
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<td>2. Transfer 250 µL of the sample into a clean 1.5 mL tube.</td>
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<td>3. Add 1250 units of Ready-Lyse Lysozyme Solution (Epicentre, Cat. No. R1802M) in 5 µL of TES Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA and 100 mM NaCl).</td>
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<tr>
<td>4. Incubate overnight at 37°C water bath (minimum 8 hours).</td>
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## DNA extraction steps

1. Dilute 1 µL of PK into 250 µL of 2× T and C Lysis Solution for each sample.

2. Add 250 µL of 2× T and C Lysis Solution containing the PK to each sample and mix thoroughly.

3. Incubate at 65°C for 15 minutes; vortex every 5 minutes.

4. Cool the samples to 37°C and add 2 µL of 5 µg/mL RNAse A to the sample; mix thoroughly.

5. Incubate at 37°C for 30 minutes.

6. Place the samples on ice for 3-5 minutes.

7. Add 250 µL of MPC Protein Precipitation Reagent to 500 µL of lysed sample and vortex vigorously for 10 seconds.

8. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 × g in a microcentrifuge. If the resultant pellet is clear, small, or loose, add an additional 25 µL of MPC Protein Precipitation Reagent, mix and pellet the debris again.

9. Transfer the supernatant to a clean 2 mL microcentrifuge tube and discard the pellet.

10. Add 850 µL of isopropanol to the recovered supernatant. Invert the tube 30-40 times.

11. Pellet the DNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.

12. Carefully pour off the isopropanol without dislodging the DNA pellet.

13. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipette.

14. Resuspend the DNA in 50 µL of TE Buffer.