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

Sample prep steps
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. *Recommended PK: Epicentre PK (Cat. No. MPRK092) or QIAGEN® Protease (Cat. No. 19155)
2. Transfer 250 µL of the sample into a clean 1.5 mL tube.
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).
4. Incubate overnight at 37°C water bath (minimum 8 hours).

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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.

Technical support is available Monday to Friday (9h00 to 17h00 ET):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com

† Epicentre MasterPure™ Complete DNA and RNA Purification Kit (Cat. No. MC85200), Version 110•9/2010.

OMNIgene•ORAL (OMR-110 and OMR-120) and OMNIgene•VAGINAL (OMR-130) are for research use only, not for use in diagnostic procedures.
Some DNA Genotek products may not be available in all geographic regions.

All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.

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