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. R1804M/R1810M, available through Lucigen®)
- 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. R1804M/R1810M, available through Lucigen) in 5 µL of TES Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA and 100 mM NaCl).
4. Incubate overnight in a 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/µL 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.

PD-PR-00554 Issue 3/2020-04