OMNIgene®•ORAL

OMNIgene®•ORAL (OM-505) DNA purification protocol using Epicentre MasterPure™ Complete DNA and RNA Purification Kit

This laboratory protocol is used for the preparation of an oral sample collected and stabilized in OMNIgene•ORAL (OM-505) for subsequent extraction of microbial DNA using the Epicentre MasterPure Complete DNA and RNA Purification Kit.

Required reagents

- Ready-Lyse™ Lysozyme Solution (Epicentre, Cat. No. R1802M)
- TES Buffer
- Epicentre MasterPure™ Complete DNA and RNA Purification Kit (Cat. No. MC85200)
  - Refer to the manual in the kit for detailed extraction protocol

Equipment required

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

Procedure

Sample preparation steps
1. When samples are received in the lab, shake very vigorously for 10 seconds.
2. Prior to purification, incubate the entire sample in the original vial at 50°C for 1 hour in a water bath or for 2 hours in an air incubator. **Note:** Incubation may be performed any time between sample receipt and extraction. This step does not need to be repeated for extraction of subsequent aliquots.
3. Transfer 250 µL of the sample into a clean 1.5 mL tube.
4. 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).
5. Incubate overnight in a 37°C water bath (minimum 8 hours).

Extraction steps
1. Dilute 1 µL of PK into 250 µL of 2× T and C Lysis Solution for each sample. Vortex to mix.
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.

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Not available for clinical diagnostic use in the United States.

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Extraction steps
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 pipet.
14. Resuspend the DNA in 50 µL of TE Buffer.

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

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: +1.613.723.5757, option 6
- Email: support@dnagenotek.com

† MoBIO PowerMicrobiome™ RNA Isolation Kit (26000-50), Version 05302013.

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