

OMNIgene®•ORAL (OM-501, OM-505) manual purification of microbial DNA using DNA Genotek's prepIT®•L2P DNA extraction kit

This laboratory protocol is used for the preparation of saliva samples collected and stabilized in OMNIgene•ORAL OM-501 and OM-505 for subsequent extraction of microbial nucleic acids.

Required reagents

- prepIT•L2P (DNA Genotek, catalog #: PT-L2P)
- RNase (10 mg/mL)
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%)
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Procedure

Purification 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 500 µL of the mixed sample to a 2 mL screw microcentrifuge tube containing glass beads.

Note: Use the equivalent of 75 μ L of 105-150 μ m glass beads. Otherwise follow bead beater manufacturer recommendations.

- 4. Using a bead beater give a 60 seconds pulse.
- 5. Add 20 µL of prepIT•L2P to the microcentrifuge tube and mix by vortexing for a few seconds.
- 6. Incubate on ice for 10 minutes.
- 7. Centrifuge at room temperature for 5 minutes at 13,000 rpm $(15,000 \times g)$.
- 8. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard the pellet containing impurities.
- 9. Add 5 µL of RNase (at 10mg/mL) and incubate the sample for 10 minutes at 37°C.
- 10. To 500 µL of supernatant, add 1 mL of cold 95% to 100% ethanol. Mix gently by inversion 10 times.
- 11. Incubate the sample at -20°C for at least 30 minutes to allow the DNA to fully precipitate.

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Purification steps

- 12. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 13,000 rpm (15,000 \times g).
- 13. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.
- 14. Carefully add 250 μL of 70% ethanol. Let stand at room temperature for 1 minute. Completely remove the ethanol without disturbing the pellet.
- 15. Add 100 µL of TE solution to dissolve the DNA pellet. Vortex for at least 5 seconds.

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

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

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