OMNIgene™•SALIVA | prepIT™•L2P
DNA and RNA

The purification of human genomic DNA from OMNIgene™•SALIVA DNA and RNA (OMR-610) using prepIT™•L2P reagent

For the purification of human genomic DNA (gDNA) from saliva collected using OMNIgene™•SALIVA DNA and RNA (OMR-610) collection kits.

The following step-by-step protocol describes how to purify gDNA from a 500 µL aliquot of sample using the prepIT™•L2P (DNA Genotek, Cat. No. PT-L2P) extraction reagent.

Equipment and reagents

- prepIT™•L2P reagent (DNA Genotek, Cat. No. PT-L2P)
- Microcentrifuge capable of running at 15,000 x *g*
- 1.5 mL microcentrifuge tubes
- Air or water incubator at 50°C
- 95%-100% ethanol (at room temperature)
- 75% ethanol (at room temperature)
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Procedure

Purification steps	Notes
1. Mix the sample collected in the OMNIgene™•SALIVA DNA and RNA kit by inverting and gently shaking for a few seconds.	• This step is to ensure that viscous samples are properly mixed.
2. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours. **Note:** The use of an air incubator may be preferable since the sample tubes may float in a water bath. If a water bath must be used, ensure the sample-containing portion of the tube remains immersed in water.	• This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated. • This incubation step may be performed at any time after the sample is collected and before it is purified. • The entire sample must be incubated in the original collection tube before aliquoting to ensure sample homogeneity. • For convenience, the sample may be incubated at 50°C overnight. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.
3. Transfer 500 µL of the mixed sample to a 1.5 mL microcentrifuge tube.	• The remainder of the sample can be stored at room temperature or frozen (-15°C to -20°C).
4. For 500 µL of sample, add 20 µL (1/25th volume) of PT-L2P to the microcentrifuge tube and mix by vortexing for a few seconds.	• The sample will become turbid as impurities and inhibitors are precipitated.
5. Incubate on ice for 10 minutes.	• Room temperature incubation can be substituted but will be slightly less effective in removing impurities.

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Purification steps	Notes
6. Centrifuge at room temperature for 5 minutes at 15,000 × *g*.	• To reduce the turbidity (A_{320}) of the final DNA solution, a longer period of centrifugation (up to 15 minutes) may be beneficial.
7. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. **Discard the pellet containing impurities.**	• The pellet contains turbid impurities. If accidentally disturbed, the tube should be recentrifuged.
8. To 500 µL of supernatant, add 600 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.	• During the mix with ethanol, the DNA will be precipitated. This may appear as a clot of DNA fibers or as a fine precipitate, depending upon the amount of DNA in the sample. • Even if no clot is seen, DNA will be recovered by carefully following the next steps.
9. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
10. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 15,000 × *g*.	• For example, place each tube in the microcentrifuge with the hinge portion of the cap pointing away from the center of the rotor. After centrifugation, the position of the pellet can be located (even if too tiny to be easily visible), it will be at the tip of the tube below the hinge.
11. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.	• This pellet contains DNA. Loss of the pellet will result in loss of the DNA. • Rotating the tube such that the pellet is on the upper wall will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant. • The supernatant may contain impurities and should be removed as completely as possible. • Excessive drying of the pellet can make the DNA more difficult to dissolve.
12. Ethanol wash: Carefully add 250 µL of 70% ethanol. Let stand at room temperature for 1 minute. **Completely remove the ethanol without disturbing the pellet.**	• **It is important to remove all ethanol from the sample. Carryover of ethanol may impact the performance of the assay.** • Take care not to disturb the DNA pellet. • The DNA pellet may be small. • Should the pellet detach, centrifuge the sample for 5 minutes at 15,000 x *g*. • After removing the 70% ethanol, the tube can be pulse-spun to allow removal of residual ethanol.

Purification steps	Notes
13. Add 100 uL of TE solution to dissolve the DNA pellet (see Equipment and reagents). Vortex for at least 5 seconds.	• If a higher concentration of DNA is desired, 50 µL of TE should be used.
14. To ensure complete rehydration of the DNA (pellet and smear) incubate at room temperature overnight followed by vortexing or at 50°C for 1 hour with occasional vortexing.	• Large amounts of high molecular weight DNA can be slow to hydrate (dissolve) completely. • Incomplete rehydration of DNA can lead to inaccuracy in estimating DNA concentration and the potential failure of downstream applications such as PCR.
15. Options for storage of the fully rehydrated DNA: a) **Recommended:** In TE, in aliquots at -20°C for long-term storage, or b) In TE at 4°C for up to 2 months.	

Technical support is available Monday to Friday (9:00 a.m.-5:00 p.m. ET):

- Toll-free (North America): 1.866.813.6354, option 6
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