

Oragene[®]/saliva sample[†] purification using the Puregene[®] DNA purification kit

DNA purification protocol for 4 mL saliva samples

Cell lysis

1. Incubate Oragene/saliva samples at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.
2. Transfer 4 mL of lysate sample (2 mL saliva plus 2 mL Oragene solution) to a 15 or 50 mL centrifuge tube.
3. Add 1 mL of cell lysis solution and 25 µL Gentra RNase A Solution (4 mg/mL). Vortex at high speed for 10 seconds to mix sample and incubate 10 minutes at room temperature.

Protein precipitation

4. Add 1.67 mL of protein precipitation solution to the cell lysate.
5. Vortex vigorously at high speed for 20 seconds to mix the Protein precipitation solution uniformly with the cell lysate.
6. Incubate the samples on ice for 5 minutes.
7. Centrifuge at 2,000 × g for 5 minutes. The precipitated proteins will form a tight dark brown pellet. If the protein pellet is not tight, repeat Step 4 followed by incubation on ice for 5 minutes and then repeat Step 5.

DNA precipitation

8. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 15 or 50 mL tube containing 5 mL of 100% Isopropanol (2-propanol) and 40 µL of Gentra Glycogen Solution (20 mg/mL).
9. Mix the sample by inverting gently 50 times.
10. Centrifuge at 2,000 × g for 3 minutes; the DNA will be visible as a small white pellet.
11. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 5 mL of 70% ethanol and invert tube several times to wash the DNA pellet.
12. Centrifuge at 2,000 × g for 1 minute. Carefully pour off the ethanol. Pellet may be loose so pour slowly and watch pellet.
13. Invert and drain the tube on clean absorbent paper and allow the sample to air dry for 5 to 10 minutes.

† Saliva samples were collected with Oragene[®]-DNA or Oragene[®]-DISCOVER.

DNA hydration

14. Add 400 μL of DNA hydration solution (400 μL will give a concentration of 200 $\mu\text{g}/\text{mL}$ if the total yield is 80 μg DNA).
15. Rehydrate DNA by incubating at 65°C for 1 hour and overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
16. For storage, sample may be centrifuged briefly and then transferred to a 1.5 mL tube. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.

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

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

Oragene®-DNA is not available for sale in the United States.

Oragene®-DISCOVER is for research use only, not for use in diagnostic procedures.

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

