ORAcollect®•Dx

Laboratory protocol for DNA extraction from ORAcollect®•Dx (OCD-100) using QIAamp® DNA mini kit

Equipment and reagents to be supplied by the user:

- QIAamp DNA mini kit (QIAGEN® Cat. no. 51304) and required reagents for *DNA purification from blood or body fluids* (spin protocol) procedure.

Procedure

1. Prior to purification, incubate the entire sample in the original ORAcollect•Dx tube at 50°C for a minimum of 2 hours in an air incubator.
2. Follow the *DNA purification from blood or body fluids* (spin protocol) procedure in the QIAamp DNA mini kit handbook (QIAGEN Cat. No. 51304), starting at step 1.
3. Changes to the protocol: In step 11, elute samples in 50 µL Buffer AE.
4. The brief version of the procedure is below for reference.

Purification steps	Notes
1. Pipet 20 µL QIAGEN Protease (or proteinase K) into the bottom of a 1.5 mL microcentrifuge tube.	
2. Add 200 µL sample to the microcentrifuge tube.	
3. Add 200 µL Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.	To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. **Note:** Do not add QIAGEN Protease or proteinase K directly to Buffer AL.
4. Incubate at 56°C for 10 minutes.	DNA yield reaches a maximum after lysis for 10 minutes at 56°C. Longer incubation times have no effect on yield or quality of the purified DNA.
5. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.	
6. Add 200 µL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.	

Purification steps	Notes
7. Carefully apply the mixture from step 6 to the QIAamp mini spin column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6,000 × *g* (8,000 rpm) for 1 minute. Place the QIAamp mini spin column in a clean 2 mL collection tube (provided), and discard the tube containing the filtrate.*	Close each spin column to avoid aerosol formation during centrifugation. Centrifugation is performed at 6,000 × *g* (8,000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp mini spin column is empty.
8. Carefully open the QIAamp mini spin column and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6,000 × *g* (8,000 rpm) for 1 minute. Place the QIAamp mini spin column in a clean 2 mL collection tube (provided), and discard the collection tube containing the filtrate.*	
9. Carefully open the QIAamp mini spin column and add 500 µL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed 20,000 × *g* (14,000 rpm) for 3 minutes.	
10. Place the QIAamp mini spin column in a new 2 mL collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.	This step helps to eliminate the chance of possible Buffer AW2 carryover.
11. Place the QIAamp mini spin column in a clean 1.5 mL microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp mini spin column and add 50 µL Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 minute and then centrifuge at 6,000 × *g* (8,000 rpm) for 1 minute.	

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 of QIAamp DNA mini kit protocol for safety information.

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

- Toll-free (North America): 1.866.813.6354, option 6
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PD-PR-00668 Issue 2/2018-10