

Protocol: Isolation of Genomic DNA from Laser-Microdissected Tissues

This protocol is for isolation of DNA using the QIAamp DNA Micro Kit from laser-microdissected tissue. Laser-microdissected tissue specimens present a particular challenge for molecular analysis, as nucleic acids must be purified from very small amounts of starting material. In addition, fixation and staining steps may compromise the integrity of DNA, and it may be necessary either to modify fixation protocols or to use cryosections from flash-frozen specimens to minimize this problem.

A wide range of equipment and consumables for sectioning, staining, and microdissection of specimens is available from Leica (www.leica-microsystems.com).

Important point before starting

- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AE or water for elution to room temperature.
- Heat a thermomixer or heated orbital incubator to 56°C for use in step 3.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.
- Carrier RNA can be added to Buffer AL if DNA is isolated from a small number of cells (see page 14).

Procedure

1. **Add 15 μ l Buffer ATL to a laser-microdissected sample collected in a 0.2 ml microcentrifuge tube (not provided).**
2. **Add 10 μ l of Proteinase K and mix by pulse-vortexing for 15 s.**
3. **Incubate the sample at 56°C for 3 h (16 h for formalin-fixed tissues), with occasional agitation of the tube. The incubation time may vary depending on the amount of tissue collected.**
4. **Add 25 μ l Buffer ATL to increase the working volume.**
5. **Add 50 μ l of Buffer AL, close the cap, and mix by pulse-vortexing for 15 s.**

In order to ensure efficient lysis, it is essential that the sample and Buffer AL are thoroughly mixed to yield a homogeneous solution.

- 6. Add 50 μ l of ethanol (96–100%) to the sample, close the cap, and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate for 5 min at room temperature.**

Note: In case room temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.

- 7. Briefly spin the 0.2 ml tube to remove drops from the inside of the lid.**
- 8. Carefully apply all of the lysate from step 7 onto the QIAamp MinElute Column without wetting the rim, close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

If the lysate has not completely passed through the column after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute Column is empty.

- 9. Carefully open the QIAamp MinElute Column and add 500 μ l Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**
- 10. Carefully open the QIAamp MinElute Column and add 500 μ l Buffer AW2 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the collection tube containing the flow-through.**

Contact between the QIAamp MinElute Column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute Column. Careless removal of the QIAamp MinElute Column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp MinElute Column.

- 11. Place the QIAamp MinElute Column in a clean 2 ml collection tube. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.**

Ethanol carryover from Buffer AW2 may cause problems in some downstream applications.

12. Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube with the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–30 μ l Buffer AE or distilled water to the center of the membrane. Close the lid and incubate at room temperature for 1 min.

If high pH or EDTA may affect sensitive downstream applications, use water for elution (see page 10).

Important: Ensure that the elution buffer is equilibrated to room temperature. If elution is performed in small volumes (<50 μ l) the elution buffer must be dispensed onto the center of the membrane for complete elution of bound DNA.

Elution volume is flexible and can be adapted according to the requirements of the downstream application. Remember that the recovered eluate volume will be up to 5 μ l less than the elution volume applied onto the column.

13. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.

Incubating the QIAamp MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.