

**User-developed
protocol**

User-Developed Protocol:

Purification of genomic DNA from cultured cells using the QIAamp[®] DNA Micro Kit

This procedure has been adapted by customers and is for purification of total DNA from cultured cells using the QIAamp DNA Micro Kit. **QIAGEN has not verified the performance of this user-developed protocol.**

IMPORTANT: Please read the *QIAamp DNA Micro Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.

Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Ethanol (96–100%)*
- 1.5 ml microcentrifuge tubes
- Pipets and pipet tips (to avoid cross contamination, we recommend pipet tips with aerosol barriers)
- Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C and 70°C
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Vortexer

Important points before starting

- Lysis time will vary depending on the size and density of the source material. The lysis conditions given here are intended to serve as guidelines.
- All centrifugation steps are performed at room temperature (15–25°C).

Things to do before starting

- Heat a water bath or heating block to 56°C.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions in the *QIAamp DNA Micro Handbook*.

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Procedure

1. **Pipet 1–100 µl media containing the cells into a 1.5 ml microcentrifuge tube (not provided).**
2. **Add Buffer ATL to a final volume of 100 µl.**
3. **Add 10 µl proteinase K.**
4. **Add 100 µl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s.**

To ensure efficient lysis, it is essential that the sample, Buffer ATL, proteinase K, and Buffer AL are thoroughly mixed to yield a homogeneous solution.

Note: For small numbers of cells we recommend adding carrier RNA to Buffer AL (see the *QIAamp DNA Micro Handbook*). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during the heat incubation in step 5.

5. **Incubate at 56°C for 10 min.**

Note: If samples are shaken during the incubation, DNA yields can be increased.

6. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**
7. **Add 50 µl ethanol (96–100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s. Incubate for 3 min at room temperature (15–25°C).**

Note: If the ambient temperature exceeds 25°C, cool the ethanol on ice before adding to the tube.

8. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**
9. **Carefully transfer the entire lysate from step 8 into the QIAamp MinElute[®] column without wetting the rim. Close the lid, 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 membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute column is empty.

10. **Carefully open the QIAamp MinElute column and add 500 µl Buffer AW1 without wetting the rim. Close the lid 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.**
11. **Carefully open the QIAamp MinElute column and add 500 µl Buffer AW2 without wetting the rim. Close the lid 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.**

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.

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- 12. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min to dry the membrane completely.**

This step is necessary, since ethanol carryover into the eluate may cause problems in some downstream applications.

- 13. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20–100 µl Buffer AE or distilled water to the center of the membrane.**

If high pH or EDTA may affect sensitive downstream applications, use water for elution (see the *QIAamp DNA Micro Handbook*).

Important: Ensure that the elution solution is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense the elution solution onto the center of the membrane to ensure complete elution of bound DNA.

QIAamp MinElute columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

- 14. Close the lid and incubate at room temperature (15–25°C) for 1 min. 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.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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Purification of genomic DNA from cultured cells using the QIAamp DNA Micro Kit (QA43 Jul-10) page 3 of 3