



QIAGEN Supplementary Protocol:

Purification of DNA from epithelial cells mixed with sperm cells using the QIAamp[®] DNA Micro Kit

This protocol is designed for purification of total (genomic and mitochondrial) DNA from fabrics or swabs containing epithelial cells mixed with sperm cells using the QIAamp DNA Micro Kit.

IMPORTANT: Please read the *QIAamp DNA Micro Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

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 or 2 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for elution steps) (available from Brinkmann [Safe-Lock, cat. no. 022363204], Eppendorf [Safe-Lock, cat. no. 0030 120.086], or Sarstedt [Safety Cap, cat. no. 72.690])*
- Pipet tips (to avoid cross contamination, we recommend pipet tips with aerosol barriers)
- Thermomixer, heated orbital incubator, heating block, or water bath
- Microcentrifuge with rotor for 2 ml tubes
- Vortexer
- Scissors
- 1 M dithiothreitol (DTT). This solution can be made in advance, and stored at –20°C in appropriate aliquots. A thawed aliquot should be discarded after use.
- **Optional:** QIAshredder spin columns (cat. no. 79654 or 79656; to harvest lysate remaining in the swab or fabric)

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see the *QIAamp DNA Micro Handbook*).

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Things to do before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in steps 3 and 10, and a second thermomixer or heated orbital incubator to 70°C for use in step 13. If thermomixers or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions in the *QIAamp DNA Micro Handbook*.

Procedure

- 1. Place the swab or a piece of fabrics ($\leq 0.5\text{cm}^2$) in a 2 ml microcentrifuge tube (not provided).**

Separate the cotton or DACRON® swab from its shaft by hand or using scissors.
- 2. Add 20 μl proteinase K and 500 μl Buffer ATL to the sample. Close the cap and mix by pulse-vortexing for 10 s.**
- 3. Place the 2 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for at least 1 h.**

If using a heating block or water bath, vortex the tube for 10 s every 10 min to improve lysis.
- 4. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.**
- 5. Remove the solid material from the tube.**

Note: Up to 200 μl of lysate remains in the swab or fabric. To harvest this remaining lysate, place the swab or fabric in a QIAshredder spin column (not supplied), place the QIAshredder spin column containing the solid material in the 2 ml tube containing the lysate, and centrifuge at full speed (20,000 $\times g$; 14,000 rpm) for 2 min. Remove and discard the QIAshredder spin column containing the solid material.
- 6. Centrifuge the tube for 5 min at full speed. Carefully transfer all but 30 μl of the supernatant to a new tube without disturbing the pellet.**

Note: For isolation of DNA from epithelial cells, transfer 300 μl of the supernatant into a 2 ml microcentrifuge tube and continue with step 12.
- 7. Resuspend the pellet in 500 μl Buffer ATL. Close the lid and mix by pulse-vortexing for 10 s. Centrifuge the tube for 5 min at full speed. Carefully aspirate and discard all but 30 μl of the supernatant without disturbing the pellet.**
- 8. Repeat step 7 at least three times.**

Note: The ratio of epithelial cells to sperm cells influences the number of repeats needed for purification of sperm nuclei.
- 9. Add 300 μl Buffer ATL, 10 μl proteinase K, and 10 μl 1 M DTT to the pellet. Close the lid and mix by pulse-vortexing for 10 s.**

- 10. Place the 2 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for at least 1 hour.**

If using a thermoblock or waterbath, vortex the tube for 10 s every 10 min to improve lysis.

- 11. Briefly centrifuge the tube to remove drops from the inside of the lid.**

- 12. Add 300 µl Buffer AL, close the lid, and mix by pulse-vortexing for 10 s.**

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

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 incubation in step 13.

Note: If carrier RNA is required (see the *QIAamp DNA Micro Handbook*), add 1 µg dissolved carrier RNA to 300 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

- 13. Place the tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 10 min.**

If using a heating block or water bath, vortex the tube for 10 s every 3 min to improve lysis.

- 14. Centrifuge the tube at full speed (20,000 x g; 14,000 rpm) for 1 min.**

- 15. Carefully transfer the supernatant from step 14 to the QIAamp MinElute[®] column without wetting the rim.**

- 16. 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.

- 17. 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.**

- 18. 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. Take care when removing the QIAamp MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column.

- 19. 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 interfere with some downstream applications.

- 20. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20–50 μ l Buffer AE or distilled water to the center of the membrane.**

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

Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). Dispense Buffer AE or distilled water 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 μ l less than the volume of elution solution applied to the column.

- 21. 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.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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