

Protocol: DNA Purification from Blood or Body Fluids (Spin Protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge. For total DNA purification using a vacuum manifold, see “Protocol: DNA Purification from Blood or Body Fluids (Vacuum Protocol)” on page 29.

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- Use carrier DNA if the sample contains <10,000 genome equivalents (see page 17).
- 200 µl of whole blood yields 3–12 µg of DNA. Preparation of buffy coat (see page 18) is recommended if a higher yield is required.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Heat a water bath or heating block to 56°C for use in step 4.
- Equilibrate Buffer AE or distilled water to room temperature for elution in step 11.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 16.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

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. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10⁶ lymphocytes in 200 µl PBS.**

If the sample volume is less than 200 µl, add the appropriate volume of PBS.

QIAamp Mini spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

Note: It is possible to add QIAGEN Protease (or proteinase K) to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

3. Add 200 μ l Buffer AL to the sample. Mix by pulse-vortexing for 15 s.

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

If the sample volume is larger than 200 μ l, increase the amount of QIAGEN Protease (or proteinase K) and Buffer AL proportionally; for example, a 400 μ l sample will require 40 μ l QIAGEN Protease (or proteinase K) and 400 μ l Buffer AL. If sample volumes larger than 400 μ l are required, use of QIAamp DNA Blood Midi or Maxi Kits is recommended; these can process up to 2 ml or up to 10 ml of sample, respectively.

Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

4. Incubate at 56°C for 10 min.

DNA yield reaches a maximum after lysis for 10 min 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 μ l ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.**

If the sample volume is greater than 200 μ l, increase the amount of ethanol proportionally; for example, a 400 μ l sample will require 400 μ l of ethanol.

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 6000 x g (8000 rpm) for 1 min. 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 6000 x g (8000 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.

Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

8. Carefully open the QIAamp Mini spin 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 Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*

It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200 μ l.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 for safety information.

- 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 $\times g$; 14,000 rpm) for 3 min.
- Recommended:** 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 min.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

- 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 200 μl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 $\times g$ (8000 rpm) for 1 min.

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

A second elution step with a further 200 μl Buffer AE will increase yields by up to 15%.

Volumes of more than 200 μl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

Elution with volumes of less than 200 μl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield (see Table 5, page 25). For samples containing less than 1 μg of DNA, elution in 50 μl Buffer AE or water is recommended. Eluting with 2 \times 100 μl instead of 1 \times 200 μl does not increase elution efficiency.

For long-term storage of DNA, eluting in Buffer AE and storing at –30 to –15°C is recommended, since DNA stored in water is subject to acid hydrolysis.

A 200 μl sample of whole human blood (approximately 5×10^6 leukocytes/ml) typically yields 6 μg of DNA in 200 μl water (30 ng/ μl) with an A_{260}/A_{280} ratio of 1.7–1.9.

For more information about elution and how to determine DNA yield, purity, and length, refer to pages 24–25 and Appendix A, page 50.