

Standard Operating Procedure IX: DNA Isolation from Whole Blood

**** NOTE: The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.**

DNA isolation will be performed using the QIAamp DNA mini kit from QIAGEN (Cat no. 51304). This protocol is a summary of the one given with the kit. Please refer to vendor's protocol for additional information. If individual investigator wishes another method to be used, take detailed notes.

Important points before starting:

- All centrifugation steps are carried out at room temperature.
- Use carrier DNA if the sample contains less than 10 000 genome equivalents.
- 200 μ l of whole blood yields 3 to 12 μ g of DNA. Preparation of buffy coat is recommended if a higher yield is required.

Things to do before starting:

- Equilibrate samples to room temperature.
- 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 17.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

PROCEDURE:

1. Pipette 20 μ l QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.
2. Add 200 μ l sample to the microcentrifuge tube. Use up to 200 μ l whole blood, plasma, serum, buffy coat or body fluids, or up to 5×10^6 lymphocytes in 200 μ l of PBS.
 - a. If RNA-free genomic DNA is required, 4 μ l of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of buffer AL.
3. Add 200 μ l of Buffer AL to the sample. Mix thoroughly by pulse-vortexing for 15 sec.
4. Incubate at 56°C for 10 minutes.
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 seconds. After mixing, briefly centrifuge the 1.5ml microcentrifuge tube to remove drops from the inside of the lid.
7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim.

8. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. 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. (N.B. When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.)
9. Place the QIAamp Mini spin column in a clean 2 ml collection tube and discard the tube containing the filtrate.
10. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim.
11. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute.
12. Place the QIAamp Mini spin column in a clean 2 ml collection tube and discard the collection tube containing the filtrate.
13. Carefully open the QIAamp Mini spin column and add 500 µl of Buffer AW2 without wetting the rim.
14. Close the cap and centrifuge at full speed (20 000 x g or 14 000 rpm) for 3 minutes.
15. Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate.
16. Centrifuge at full speed for 1 minute.
17. Place the QIAamp mini spin column in a clean, properly identified 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate.
18. Carefully open the QIAamp Mini spin column and add 200 µl of Buffer AE or distilled water.
19. Incubate at room temperature for 5 minutes.
20. Centrifuge at 6000 x g for 1 minute.
21. Discard the column and close the cap of microcentrifuge tube.
22. Store the sample at -80°C until use.

For complete information, refer to the protocol "QIAamp DNA Mini and Blood Mini Handbook", version of November 2007, p.27-29.