DNA Purification from Plasma (Spin Protocol).v1

This is for purification of total (genomic and mitochondrial) DNA from Plasma using a microcentrifuge according to QIAamp® DNA Mini and Blood Mini Kit (50) Handbook.

Procedure

1. Equilibrate samples to room temperature (RT).
2. Heat a heating block to 56°C.
3. Equilibrate Buffer AE to RT.
4. Pipet 20 µl QIAGEN Protease into each of the 1.5-ml microcentrifuge tubes.
5. Add 200 µl plasma to the microcentrifuge tube. Note: If adding QIAGEN Protease (or proteinase K) to samples, make ensure to properly mix after adding the enzyme.
6. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s. Note: DO NOT add QIAGEN Protease or proteinase K directly to Buffer AL.
7. 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.
8. Briefly spin the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
9. Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. Briefly spin the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
10. Carefully apply the mixture from above step to the QIAamp Mini spin column (the column) in a 2 ml collection tube without wetting the rim. Spin at 6,000 x g (8,000 rpm using Eppendorf Centrifuge 5424) for 1 min. Place the column in a clean 2 ml collection tube (provided) and discard the tube containing the filtrate. If the lysate has not completely passed through the column, spin again at higher speed until the column is empty.
11. Add 500 µl Buffer AW1 to the column without wetting the rim. Sping at 6,000 x g (8,000 rpm) for 1 min. Place the column in a clean 2 ml collection tube (provided) and discard the collection tube containing the filtrate.
12. Add 500 µl Buffer AW2 to the column without wetting the rim. Spin at full speed (20,000 x g; 14,000 rpm) for 3 min and discard the collection tube with the filtrate.
13. Place the column in a new 2 ml collection tube (not provided) and spin at full speed for 1 min to eliminate the chance of possible Buffer AW2 carryover. Discard the collection tube containing the filtrate.
14. Place the column in a clean 1.5 ml microcentrifuge tube (not provided). Add 50 µl Buffer AE (provided) to the column and incubate at room temperature (15–25°C) for 5 min. Spin at 6,000 x g (8,000 rpm) for 1 min.
15. Store the eluted DNA in Buffer AE at –20°C.

Preparation of reagents

QIAGEN Protease stock solution (store at 2–8°C or -20°C)

Pipet 1.2 ml protease solvent (provided) into the vial containing lyophilized QIAGEN Protease. Stable for 2 month at 2-8°C. Long storage should be at -20°C.

Buffer AL (store at room temperature, 15–25°C)

Mix Buffer AL thoroughly by shaking before use. If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C. Stable for 1 year.

Buffer AW1 (store at room temperature, 15–25°C)
Buffer AW1 is supplied as a concentrate (19 ml). Before using **for the first time**, add 25ml ethanol (96–100%) to obtain 44ml Buffer AW1 and mark it "Ethanol added" and date it. Stable for 1 year.

Buffer AW2 (store at room temperature, 15–25°C)
Buffer AW2 is supplied as a concentrate (13 ml). Before using **for the first time**, add 30 ml ethanol (96–100%) to obtain 43ml Buffer AW2 and mark it "Ethanol added" and date it. Stable for 1 year.