Purification of free fetal DNA from maternal plasma with QIAamp DSP Virus Kit

1. Purpose

For isolation and purification of circulating nucleic acids from 500 µl of EDTA-treated plasma.

2. Reagents

QIAamp DSP Virus Kit (order no. 60704), Qiagen, Hilden, Germany
Protease (order no. 19155, Qiagen)
Ethanol (96-100%)
Aq. Dest.
Collection Tubes (2ml, order no. 19201, Qiagen)

3. Instruments

Centrifuge
Microcentrifuge
Heating block

4. Procedure

Things to do before starting

- Separate Plasma from cells by centrifugation at low speed, 2,500xg for 10 min without brake.
- Separate Plasma from cell fragments by centrifugation at 18,000xg for 45 min.
- Equilibrate samples to room temperature (15–25℃), and ensure that they are well mixed. In case cryoprecipitates are visible, spin down the precipitates in a table top centrifuge at full speed.
- Set heating block to 56℃.
- Place Ethanol on ice.

1. Pipet 100 µl QIAGEN Protease (QP) into a Lysis Tube (LT)
2. Add 500 µl plasma or serum to the Lysis Tube (LT). Close the lid of the LT and pulse vortex for 10-15 sec to mix sample with protease.
3. Add 500 µl Lysis Buffer (AL) to the Lysis Tube (LT), close the lid, and mix by pulse-vortexing for 15 seconds.
4. To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogeneous solution.
5. Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).
6. Incubate at 56℃ (±1℃) for 20 min (±1 min).
7. Centrifuge the Lysis Tube (LT) for ≥5 s at full speed to remove drops from the inside of the lid.
8. Change gloves and open the Lysis Tube (LT) carefully.
9. Add 600 µl ethanol (96–100%) to the Lysis Tube (LT), close the lid, and mix thoroughly by pulse-vortexing for ≥15 s. Incubate for 10 min (±1 min) at room temperature (15–25℃).
10. Centrifuge the Lysis Tube (LT) for ≥5 s at full speed to remove drops from the inside of the lid.
11. Carefully apply 570 µl the mixture from step 7 to the QIAamp Column (in a 2 ml collection tube) without wetting the rim, close the cap and centrifuge at 6,000xg (8,000rpm) for 1 minute. Place the QIAamp Column in a clean 2 ml collection tube, and discard the tube containing the filtrate. Repeat this step 2 times.
12. Carefully open the QIAamp Column and add 600 µl Wash Buffer 1 (AW1) without wetting the rim, close the cap and centrifuge at 6,000xg (8,000rpm) for 1 minute. Place the QIAamp Column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
13. Carefully open the QIAamp Column and add 750 µl Wash Buffer 2 (AW2) without wetting the rim, close the cap and centrifuge at 20,000xg (14,000rpm) for 3 minutes. Place the QIAamp Column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
14. Carefully open the QIAamp Column and add 750 µl ethanol (96–100%) to the QIAamp MinElute Column without wetting the rim. Avoid touching the QIAamp MinElute Column membrane with the pipet tip, close the cap and centrifuge at 20,000xg (14,000rpm) for 3 minutes.
15. Place the QIAamp Column in a clean 2.0 ml collection tube, and discard the tube containing the filtrate. Centrifuge at 20,000g (14,000rpm) for 1 min. Omission of the dry centrifugation might lead to inhibition of the downstream assay.

16. Place the QIAamp MinElute Column in a new Wash Tube (WT), and incubate with the lid open at 56℃ for 5 min to evaporate any remaining liquid.
17. Place the QIAamp MinElute Column in a clean Elution Tube (ET), and discard the Wash Tube (WT). Carefully open the lid of the QIAamp MinElute Column, and apply 40 or 60 µl Elution Buffer (AVE) to the center of the membrane (the elution volume depends on the volume required for downstream applications). Close the lid and incubate at room temperature (15–25℃) for 5 min (≥3 min).
18. Centrifuge at full speed (20,000xg, or 14,000 rpm) for 1 minute to elute the DNA nucleic acids.

5. Notes

To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogeneous solution. Since Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully or by using a suitable pipet such as an Eppendorf multistep pipet or equivalent. Do not add Qiagen Protease (QP) directly to Lysis Buffer (AL).

Plasma samples separated from cells and cell fragment can be stored at <-70℃

6. References

1. Liu Z, Gutensohn K, Hempel M, Legler TJ. Optimisation of the QIA-amp DSP Virus Kit for the extraction of foetal DNA from maternal plasma. Clinical Chemistry 2005;51:P73.
2. Legler TJ, Liu Z, Ait Soussan A, van der Schoot C.E. Results from the first SAFE workshop on the extraction of fetal DNA from maternal plasma. Clinical Chemistry 2005;51:O31

7. Reason for Revision

Not applicable (1st version)