Experimental Protocol

Plasma preparation for liquid biopsy studies

Total time for experiment: 40 minutes

Reagents:

4ml or 10ml Vacutainer K2 EDTA tube (Becton Dickinson, ref: # 367864 (4ml) #367525 (10ml))
Tubos Streck Cell-Free DNA BCT CE 10 ml (ref: # 218997 (100 tubes))
4ml polypropylene cryogenic vial, round bottom, self-standing (e.g. Corning, #430662) or 1ml Eppendorf tubes
10ml and 5ml serological disposable pipettes or p1000 filter tips
15ml polypropylene centrifuge tubes
Freezer storage boxes for 1-4ml cryogenic vials (e.g. Corning, #431120)

Instruments and tools:

Centrifuge, capable of ~3000g with a swing bucket rotor
Pipetting aid or plastic disposable bulb pipettes or p1000 pipette
-80^{o}C Temperature Freezer

Prior considerations:

Samples should ideally be processed within 4 hours from the time of extraction when using EDTA tubes or 24-48 if using Streck tubes.

Ideally Streck tubes should be used for blood collection as they contain a preservative that stabilizes nucleated blood cells and prevents the release of contaminating genomic DNA. In theory, cfDNA and gDNA are stable for up to 14 days at 6 °C to 37 °C after blood extraction. However, the samples should be processed within 24-48 hours in order to avoid contamination with lysed white blood cells.

Steps:

- Record the time and date of the sample extraction as well as the subject ID in an appropriately designed sample database.

- Centrifuge the EDTA tube at room temperature (15°C to 25°C) for 10 min at 1600 (± 150)g.

  N.B. sample in Streck tubes can be centrifuged at 3000g for 20 minutes.

- After centrifugation, carefully remove tube from centrifuge and transfer the plasma (supernatant) to a 15ml centrifuge tube without disturbing the cellular layer using a disposable serological pipette (or disposable bulb pipette or p1000 pipettes with filter tip). *N.B. Aspirate ~5mm above the leukocyte layer to reduce contamination of the plasma with cells.*
- Centrifuge the plasma in the 15ml centrifuge tube at room temperature (15°C to 25°C) for 10min at 3000 (±150)g to remove any residual intact blood cells carried over from the first centrifugation step.
  N.B. sample scan also be centrifuged at 3000g for 20 minutes.

- After centrifugation, carefully remove tube from centrifuge and transfer 1ml-4ml of plasma to 1-4ml polypropylene cryogenic vials using a disposable serological pipette (or disposable bulb pipette or p1000 pipettes with filter tip). *N.B. Leave a residual volume of approximately 0.3 ml (~7mm) on the bottom of the 15ml tube to avoid contaminating the plasma with cells.*

- Immediately freeze plasma upright in storage box at –80°C

- Collect the cellular lawyer (buffy coat) using a P1000 and filtered tip and transfer it to a 2 mL Eppendorf. Immediately freeze it and storage in -20C or -80C freezer.

N.B. Aliquots of an adequate volume should be used in order to avoid freeze-thawing of the samples. The volume and number of aliquots will depend on the downstream use of the samples and also the storage capacity of the -80 freezer. 1ml aliquots are useful as this is the standard volume used for many cfDNA extraction protocols and various aliquots can be defrosted if more volume is required.

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