Experimental Protocol

Platelets 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))
2 mL Eppendorf tubes
1ml or 3ml serological disposable pipettes or p1000 filter tips
15ml polypropylene centrifuge tubes
Freezer storage boxes for 1-4ml cryogenic vials (e.g. Corning, #431120)
RNAlater™ Stabilization Solution (e.g. Thermo Fisher, #AM7020)

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.

Steps:

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

- First centrifugation of EDTA tubes at low r.p.m (speeds), 120g for 20 minutes at room temperature (RT).

- After this centrifugation, transfer 9/10 of plasma (containing platelets) to the 15 mL falcon tube.

- Centrifuge the Falcon tube with the 9/10 of plasma at 360 g for 20 minutes.

- Transfer supernatant to a new 15 mL falcon tube labeled as plasma without touching the pellet with platelets.

- Resuspend the pellet of platelets with the residual plasma and transfer platelets to the 2 mL tubes with screw caps.

Add 60 to 80 µLof RNAlater solution to the tube and mix gently.

- Refrigerate tubes at 4ºC overnight and freeze at -80ºC the following day.

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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