Experimental Protocol

PBMCs 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))
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)

Red Blood Cell Lysis Buffer (RLB) 10x (Stock solution).

For 1L of RLB 10x, the following reagents are needed:

Reagents	Molar concentration	Quantity
NH4Cl	1.55 M	84 g
HEPES 1M	100 mM	100 mL
EDTA 0.5 M	10 mM	20 mL
ddH2O	-	1000

Red Blood Cell Lysis Buffer (RLB) 1x (Working solution).

For 1L of RLB 1x: 100 mL of RLB 10x
900 mL of ddH2O

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.
- Centrifuge the EDTA tube at room temperature (15°C to 25°C) for 10 min at 1600 (± 150)g.
- Transfer the supernatant (plasma) to the tube falcon labeled as Plasma.
- Using a Pasteur pipette, obtain the white layer localized between the sedimented red blood cells and the blood plasma and transfer it to the new 15 mL falcon tube. Previously, this tube should have been filled up with 12 mL of RLB 1x.
- Incubate for 30 minutes at RT in a tube roller. Then, centrifuge at 1.700 g for 10 minutes at RT.
- Discard residual blood. If the pellet is still red (containing erythrocytes), the tube should be filled up again and incubated for 10 more minutes. Centrifuge again if so.
- Wash the pellet 3 times using approximately 10 mL of RLB 1x each time.
- Resuspend the leucocyte pellet with 1 mL of RLB 1x.
- Divide the volume into the two Eppendorfs tubes (1.5 ml microcentrifuge tubes) with screw caps previously labeled.
- Centrifuge at 400g for 5 minutes at RT. Discard the supernatant. Resuspend leucocyte pellet with 2 volume of RNAlater solution. Freeze tubes at -80ºC.

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.

Contact:

liquidbiopsy@ciberonc.es