ISOLATION OF PBMC FROM HUMAN BLOOD

Equipment	Specification	Supplier
Centrifuge	Heraeus Multifuge X3R	Thermo Fisher Scientific
1000µL pipette	Monochanel Research Pipette, 100-1000µL	Eppendorf
200µL pipette	Monochanel Research Pipette, 100-1000µL	Eppendorf
100µL pipette	Monochanel Research Pipette 10-100µL	Eppendorf
10µL pipette	Monochanel Research Pipette, 2-10µL	Eppendorf
Biosafety cabinet	NA	Thermo Fisher Scientific

Material	Specification	Catalog No.	Supplier
50mL tubes	Volume up to 50mL, sterile, PP	62554502	Sarstedt
15mL tubes	Volume up to 15mL, sterile, PP	62547254	Sarstedt
pipettes 5mL	Serological, pipeting volume 1-7mL	4487	D. Dutscher
pipettes 10mL	Serological, pipeting volume 1-13mL	4488	D. Dutscher
pipettes 25mL	Serological, pipeting volume 2-35mL	4250	D. Dutscher
Plastic Pasteur pipettes	Tige etroite st unit x500	043251S	D. Dutscher
Pipette tips	Filtertips, pipeting volume 100-1000µL sterile	02-708-404	Fisher Brand
Pipette tips	Filtertips, pipeting volume 10µL, sterile	02-707-442	Fisher Brand

Reagent	Specification	Catalog No.	Supplier	Storage/validity
Ficoll	Lymphocytes separation Medium	CMSMSL01-01	Eurobio	In dark
PBS	D-PBS, 500mL, no Mg, no Ca	14190-094	Gibco	4-30°C, 36 months
Ethanol	70% (v/v)	NA	internal	15-25°C

IMPORTANT: Ficoll, PBS and centrifuge MUST be at room temperature (RT)

1. Disinfect the tubes of blood by wiping with SurfaSafe (Anios)
2. Dilute total blood with the same volume of 1x PBS (at RT)
3. Take 50ml Falcon tubes prefilled with 15ml of Ficoll and slowly layer 30ml of the diluted blood over the Ficoll being careful not to mix – two layers should be clearly separated
4. Note: For other volumes keep the ratio diluted blood:Ficoll around 2:1
5. Securely close the tube and centrifuge at 800g for 20min at RT, NO BRAKE
6. Use a sterile single use Pasteur pipette to harvest the mononuclear cell layer (ring) and transfer into a fresh 50ml tube.
7. Optional: you can discard the upper phase before recuperating the ring.
8. Add cold 1x PBS up to 50ml
9. Centrifuge at 300g for 8min at 4°C
10. Immediately flick out supernatant
11. Resuspend the pellet
12. Add 5ml cold 1x PBS over the remaining cell pellet and mix to thoroughly resuspend the cells