Isolation of Human Monocyte Directly from Blood (LRS Cone Vitalant)

Reagents and Materials:

LRS Cone, Scissor, 50ml Falcon tubes, 10ml syringe, 18G needle, 100mL of PBS (no calcium/ magnesium) containing 2% FBS and *2mM EDTA*, 50ml of PBS (no calcium/ magnesium) containing 1*mM EDTA,* 30ml of RPMI1640 with 2% FBS, Refrigerated Centrifuge (swing out bucket, 50ml holder), EasySep™ Direct Human Monocyte Isolation Kit and MACSxpress® Separator (Electromagnet).

Additional: Hemocytometer, anti-human CD14 APC (# 301808, Biolegend), anti-human CD45 Alexa488 (#304017, Biolegend), Ghost Dye™ Violet 510(# 13-0870-T500, Cytekbio) PBS, 0.5M EDTA (#15575020, Invitrogen)

Protocol:

1. LRS cone arrive on ice keep refrigerated until use (fresh cone < 24 hrs of blood collect in EDTA is ideal for myeloid cell isolation)
2. In hood, wipe (70% ethanol) both side of tubes on the cone, first use scissor to cut tube at base of cone and place it on 50ml falcon, next cut the tube at tip of cone, allow to drain and air push from tip (use 10ml syringe and 18G needle).
3. Wash cone thoroughly 3 times using 10ml of PBS (no calcium/ magnesium) containing 2% FBS and *2mM EDTA* per wash until clear base of the cone.
4. Thus, 40ml blood obtained after step3, centrifuge 800xg, 10min, 18-21°C
5. Pipet remove supernatant carefully
6. Suspend the pellet cells to 10 ml in PBS (no calcium/ magnesium) containing 2% FBS and *2mM EDTA.*
7. Add Monocyte isolation antibody cocktail (50ul/ml of blood) and Add Spherobeads, (50ul/ml of blood; vortex the beads well before addition), mix blood well with repeated pipetting and incubate at RT for 5 min.
8. Makeup volume 3x by adding 20 ml of PBS (no calcium/ magnesium) containing 1*mM EDTA*
9. Mix well and place the tube open on MACSxpress® Separator for 15mins at RT
10. Collect clear solution completely on to the new 50ml tube.
11. Add same volume of Spherobeads (as in step 7) in the tube of step10 and incubate for 5 min at RT
12. Place the tube open on MACSxpress® Separator for 10 mins at RT and collect clear solution completely on to the new 50ml tube (repeat)
13. Place the tube open on MACSxpress® Separator for 10mins at RT and collect clear solution completely on to the new 50ml tube.
14. Centrifuge at 300xg, 5 min, 4°C, remove supernatant, resuspend and wash in 20ml of RPMI1640 containing 2% FBS.
15. Centrifuge at 300xg, 5 min, 4°C, remove supernatant, resuspend in 5ml of RPMI1640 containing 2% FBS. The isolated human monocyte is ready for Count, purity and experiments.