

# Preparation of CD14+ Monocytes

## Materials:

- EDTA blood collection tubes
- 0.9% NaCl solution, sterile filtered
- Ficoll-Paque™ PLUS (GE Healthcare 17-1440-03)
- PBS (Sigma P-5368) containing 2% FCS, 1mM EDTA (Sigma E-6758), sterile filtered (=Incubation buffer)
- EasySep® Human CD14 Positive Selection Kit (StemCell 18058)
- EasySep® Magnet (StemCell 18000)
- UltraCulture (Lonza BE12-725F) containing antibiotics, 10% FCS (=Medium)
- all solutions except Ficoll-Paque™ PLUS prewarmed to 37°C
- tissue culture plastic (centrifuge tubes, dishes, flasks, ...)

## Procedure:

1. Pipette 12.5ml Ficoll-Paque™ PLUS into a sterile 50ml centrifuge tube
2. Dilute 12.5ml collected blood with 12.5ml 0.9% NaCl solution
3. Slowly overlay the Ficoll-Paque™ PLUS layer with 25ml diluted blood
4. Do not mix, tap or shake!
5. Centrifuge for 25 minutes at 1800 rpm with brake off
6. Remove the upper layer containing plasma and platelets, leaving the layer of mononuclear cells undisturbed at the interface
7. Transfer the layer of mononuclear cells to a new sterile 50ml centrifuge tube
8. Dilute/wash with at least 3 volumes of 0.9% NaCl solution
9. Count cells, calculate the total number of cells
10. Centrifuge for 10 minutes at 1800 rpm with slow brake
11. Remove supernatant, resuspend cell pellet in 1ml Incubation buffer per 10<sup>8</sup> cells
12. Transfer the cell suspension into a 5ml round-bottom tube
13. Add EasySep® Positive Selection Cocktail at 100µl/ml, mix well
14. Incubate for 15 minutes at room temperature
15. Mix EasySep® Magnetic Nanoparticles by pipetting up and down more than 5 times
16. Add EasySep® Magnetic Nanoparticles at 50 µl/ml cells, mix well
17. Incubate for 10 minutes at room temperature
18. Bring the cell suspension to a total volume of 2.5 mL by adding Incubation buffer, mix well
19. Place the tube into the EasySep® Magnet
20. Incubate for 5 minutes
21. Pick up the magnet and pour of the supernatant, leaving the tube in the magnet
22. Remove the tube from the magnet
23. Add 2.5 ml Incubation buffer, mix well
24. Place the tube back into the EasySep® Magnet
25. Incubate for 5 minutes
26. Repeat steps 21-25
27. Pick up the magnet and pour of the supernatant, leaving the tube in the magnet
28. Remove the tube from the magnet
29. Resuspend cells in 2.5ml Medium
30. Count cells, calculate the total number of cells
31. Dilute with medium and transfer resuspended cells into an appropriate tissue culture vessel
32. Use cells as soon as possible to prevent unwanted differentiation