Enrichment of PBMCs with monocytes

This protocol is used in our lab to reduce the costs of the cell sorting with MACS reagents. The cell suspension obtained after this protocol contains 40-70% monocytes. This cell suspension is than used for positive or negative MACS separation.

Buffy coats to be used for this protocol must be fresh and produced without platelet depletion. The volume of buffy coat that we obtain from Red Cross is usually about 75 ml.

1. Dilute buffy coat to 150 ml with PBS (all PBS without Ca and Mg!).
2. Prepare 2 Ficoll (Biocoll) gradients in Leucosep tubes (15 ml Ficoll per tube).
3. Apply 30 ml of diluted blood to each gradient.
4. Centrifuge 30 min at 400 x g without brakes.
5. Collect the PBMC fraction and transfer it into a 50 ml tube.
6. Fill the tube with PBS, centrifuge 10 min at 300 x g.
7. Resuspend good in 50 ml PBS, centrifuge 10 min at 200 x g.
8. Resuspend good in 50 ml PBS, centrifuge 10 min at 300 x g.
9. Prepare standard Percoll gradient (see below)
10. Apply all the cells on the Percoll gradient.
11. Centrifuge 30 min at 400 x g without brakes.
12. Collect enriched PBMCs and transfer into a 50 ml tube.
13. Fill the tube with PBS, centrifuge 10 min at 300 x g.
14. Resuspend good in 50 ml PBS, centrifuge 10 min at 200 x g.
15. Resuspend good in 50 ml PBS, centrifuge 10 min at 200 x g.
16. Determine the cell number, take an aliquot for FACS analysis and proceed with MACS separation.

Continuous Percoll gradient preparation:

Mix in 50 ml tube*	13.5 ml Percoll (Amerhsam)
	15.0 ml MEM Spinner modification (Sigma)
	1.5 ml 10x Earle's salts solution (Biochrom)
total	30.0 ml

Centrifuge 10 min at 14.000 x g without breaks in a fixed angle rotor.
Carefully take the gradient out of the rotor. The gradient is ready to use now.

*The tube should be able to resist centrifugation at 14.000 x g.