

## Isolation of monocytes from monocyte enriched PBMC fraction using CD14 magnetic beads

This protocol uses the PBMC fraction enriched in with monocytes by density gradient centrifugations (protocol may be found at [www.methods.info](http://www.methods.info)). Reduction of the amount of microbeads in comparison to Miltenyi protocol reduces the costs of the experiment.

### Protocol

Resuspend the cells in buffer (0.5 % BSA, 2 mM EDTA in PBS) at a concentration  $10^7$  cells per 90  $\mu$ l.

Add 10  $\mu$ l CD14 micro beads per  $10^7$  cells.

Incubate the suspension for 15 min at a rotator at 4°C.

Add 10 ml of the buffer to the cells and centrifuge 10 min at 300 g.

Resuspend the cell pellet in 500  $\mu$ l buffer.

Place an LS column in the magnetic separation unit (do not remove the moving part out of the unit!) and wash it with 3 ml of buffer.

Change the collecting tube and apply the cell suspension on the column.

Collect flow-through.

Wash the column 3x with 3 ml buffer collecting flow-through in the same tube.

Take the column out of the separation unit and transfer it to a new collection tube

Elute the cells with 5 ml buffer.

Count the cells and take an aliquote for FACS analysis.

Centrifuge the cells 10 min at 300 g.

Resuspend the cell pellet in X-vivo 10 medium at a concentration of  $5 \cdot 10^5$  cells/ml.

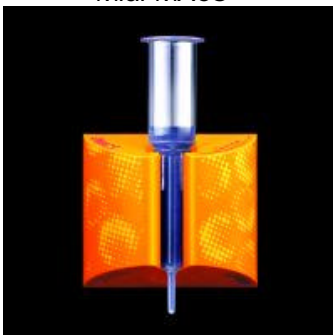
Transfer the cells to the cell culture dishes and add desired cytokines.

### Note:

PBS should be without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ !

### Separation units

Midi MACS



quadro MACS



Images were kindly provided by Miltenyi Biotec