PBMC purification using Ficoll

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REAGENTS:

- heparinated blood
- medium
- Ficoll separating solution
- Live/Dead staining solution
- freezing media

EQUIPMENT:

- pipetus
- 5 ml-, 10 ml pipettes
- 10 ml shorty-pipettes
- 50 ml Falcon tubes
- counting chamber
- 1.5 ml Eppendorf tubes
- 1,8 ml cryotubes
- „Mr. Frosty"

REAGENT SETUP:

- **medium:** RPMI 1640 medium (Cambrex, cat.no. BE12-702F/U1) -> lab name is RPMI
- **Ficoll separating solution:** density 1.077g/ml (Biochrom AG, cat.no. L6115) -> lab name is Ficoll, **keep in the dark!**
- **Live/Dead staining solution:** for the counting, Trypan Blue (Gibco, Invitrogen, Cat.no. 15250-061)
- **freezing media:** 10% DMSO (Sigma, cat.no. D2650) in FCS (Biochrom AG, cat.no. S0115), always prepare fresh and keep at 4°C
- **"Mr. Frosty":** box to freeze the cells slowly with -1°C/min

  always keep freezing media & "Mr. Frosty" at 4°C!

FICOLL

Warm up the RPMI to 37°C! Use one 50 ml Falcon tube for up to 25 ml heparinized blood!

1) Transfer the blood:

pour the heparinized blood from the blood sample tubes into 50 ml Falcon tubes

1800 rpm, 10', 21°C

during the centrifugation:

wash the blood sample tubes with RPMI and transfer in a separate 50 ml Falcon tube (labeled “wash”)

2) Plasma:

transfer the plasma (SN) of the centrifugated Falcon tubes into a fresh Falcon tube

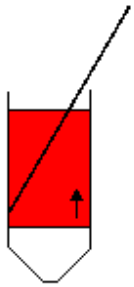
make aliquots in 1,8 ml cryotubes, store at -80°C

3) Ficoll:

add the “wash” to the 50 ml Falcon tubes and fill with RPMI to a total volume of 35 ml

mix to solve the pellet

in the previously prepared tubes overlay the Ficoll



slowly with the diluted blood

2100 rpm, 17', 21°C **NO BRAKE!!!** (ϒ0)

4) Isolation of the lymphocytes:

remove the lymphocyte ring and also the media and Ficoll with a 10 ml shorty-pipette and transfer to a new 50 ml Falcon tube (discard only the pellet)
fill up to 50 ml with RPMI

1600 rpm, 13', 21°C, discard the SN

transfer the cells (of the same individual, from multiple Falcon tubes) to a single Falcon in 25 ml RPMI

1600 rpm, 13', 21°C, discard the SN

resuspend the pellet in 25 ml RPMI

1300 rpm, 5', 21°C, discard the SN

5) Counting & freezing:

resuspend the pellet in 10 ml or 20 ml RPMI (according to the pellet)

count the cells in a counting chamber (1/2 or 1/5 in Trypan Blue)

separate the cells according to the aliquots that you want to freeze

1500 rpm, 5', 21°C, discard the SN

prepare fresh freezing media

use 1ml freezing media/cryotube

store the cryotubes in “Mr. Frosty” at -80°C for one day, then store in liquid N_2

Notes: Washing steps can be carried out in a different buffer or at a different temperature according to the experiment to be performed.

for storage: no changes

for stimulation & ICS: no changes

6) Serum collection:

Serum is obtained in a separate tube!

remove the liquid part and transfer to a 50 ml Falcon tube

2500 rpm, 20', 21°C

transfer the serum (SN) to a fresh Falcon tube

make aliquots of approx. 500 µl in 1,5 ml Eppendorf tubes, store at -20°C