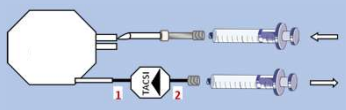
Isolation of CD34+

Preparation of solutions

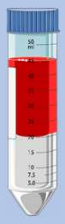
- Elution buffer: 85% PBS + 10% ACD + 5% FBS
- DGM preparation
- Dextran 2% at room temperature

Cell collection



- Transfer the eluate to a 50 mL tube
- Dilute with Dextran 2%

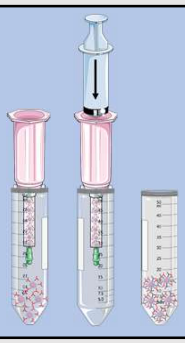
Buffy Coat



- Recover the supernatant and add it on top of the DGM
- Centrifuge at 400 g for 30 min with brake off
- Collect the PBMC fraction

- Washing steps (centrifugation 400 g for 10 min)

CD34+ selection



- Perform a cell count
- Incubate with the antibody for 30 min
- Washing step
- Pass the sample on the column (twice)

Freezing

- Perform a cell count and prepare:
  - 60% Stemspan + 40% FBS
  - 40% Stemspan + 40% FBS + 20 % DMSO
- Aliquot by 400 µL in cryotubes

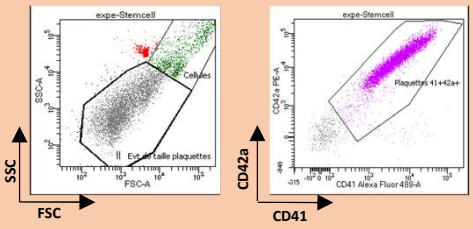
CD34⁺ Purity

- Incubate 100 µL of sample with 2 µL of hCD34-PE or IgG-PE antibody (for 30 min)
- Complete to 300 µL and perform flow cytometry analysis

Platelet Release

Release platelets

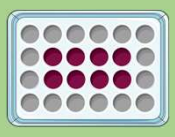
- At day 13
- 5 successive pipetting
- Perform cytometry tests



Cell Culture

Cell culture D0-D7

- Seed CD34+ medium / PSG / Human LDL / CC220 / SR1
- Incubate at 37°C for 7 days



Cell culture D7-13

- Seed cells in medium / TPO / Human LDL / SR1
- Incubate at 37°C for 6 days