Harvest and Staining Protocol Dendritic Cells (Spleen)

Pool if possible 3 female mice before sorting

Authors: Matthias Farlik (*matthias.farlik@meduniwien.ac.at*), *Thea Gorki* (anna-dorothea.gorki@gst-antivirals.com), Stephen Shoebridge (sshoebridge@outlook.com)

1) Take spleen
2) Inject with and place the spleen in a digestion mixture (RPMI (Sigma), 2% FBS, 1mg/ml Collagenase D, 20 µg/ml DNase I)
3) Incubate 30min, 37°C in a 24-well cell culture dish
4) Smash spleen over 100µm (or 70µm) strainer using syringe plunger, wash with 10-20ml DMEM/10% FCS/P/S
5) Centrifuge 5min, 500g, 4°C
6) Resuspend pellet in 1ml Red Blood Cell Lysis Solution (Promega, Z3141), incubate 5min on ice, stop reaction by adding 1xPBS up to 50ml
7) Centrifuge 5min, 500g, 4°C
8) Discard supernatant→resuspend in 1ml PBS/2%BSA
9) Filter through 70µm strainer and wash the strainer with another 1ml of PBS/BSA
10) Split sample→ e.g. unstained (10% of sample), Panel (see below for Antibodies) → transfer aliquots into FACS tubes
11) Centrifuge 5min, 500g, 4°C
12) Resuspend in 100µl PBS/2%BSA+blocking AB (1:500), incubate for 10-15min on ice
13) Directly add viability dye (1:4000)+ staining mix
    ! NO washing inbetween the Viability dye and the staining mix → staining mix is calculated per mouse, so double the amount if two mice are pooled, triple the amount if three mice are pooled, …
14) Incubate 30min, 4°C, dark
15) Add 1ml PBS/2% BSA→ Centrifuge 5min, 500g, 4°C
16) Resuspend in 300µl PBS/2% BSA→ filter over 40µm strainer (5ml Polystyrene round bottom tubes with Cell Strainer) immediately before sorting and rinse the filter with 1ml PBS/BSA

Staining mix:

Marker	Fluorochrome	Laser	Company	Dilution
Live/Dead	APC-Cy7	R	Invitrogen, 65-0865-14	1:10,000
MHCII	PE	B	Invitrogen, 12-5321-82	1:100
CD11c	PECy7	B	eBioscience, 25-0114-82	1:100

Viability Dye: Fixable Viability Dye Invitrogen, 65-0865-14
Blocking AB: Anti-mouse CD16/CD32 purified, eBioscience 14-0161-82
FACS tubes (sterile): Falcon #352058

Sorting (FACS Aria Version 2 or higher):
-use 70µm nozzle, bi-exponential setting for all axis (except SSC and FSC)
FACS tube sorting:
-sort around 20.000 events/sec
-Precision mode: Purity (or 4-way purity if sort 4 populations)
-population was sorted using inner positions (more stable)
- Sheath fluid to use: FACS FLOW

Expected yield per spleen of an 8-12 week old mouse:

Dendritic cells: 100.000 – 300.000 cells

Example Dendritic Cell gating strategy: