Protocol 608_Surface FACS protocol

Reagents:

- 10x FACS buffer:
  10x PBS powder
  add 400ml ddH_2O
  add 200ml FCS (**NOT** cell culture grade!)
  add 200ml 0.5M EDTA
  add 1g NaN_3
  fill up to 1L with ddH_2O

- 1xFACS buffer: 100ml 10x FACS buffer + 900ml ddH_2O

- 0.5M EDTA: Add 186.1 g of $EDTA \cdot 2H_2O$ to 800ml ddH_2O (if different EDTA in stock, recalculate amount for 0.5 moles using the molecular weight given on the container). Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20g of NaOH pellets!). Dispense into aliquots and sterilize by autoclaving.

- FACS tubes (BD REF352052)

Method:

1. Prepare and label a FACS tube for each sample and for each single stain control (including unstained). Use compensation beads whenever possible.

2. Wash samples one times with FACS buffer in FACS tubes. Centrifuge at 4C at 1500rpm for 5min. Pour off supernatant. Vortex tube.

3. Add antibody cocktail and single stains to the samples and single stain controls respectively. Vortex or flick tube to mix. Incubate for 20-30min at 4C in the dark (fridge or on ice).

4. Wash one times with FACS buffer. Centrifuge at 4C at 1500rpm for 5min. Pour off supernatant.

5. Optional: While vortexing add 200µl FACS Buffer + 200µl 4%PFA to fix the cells.

6. Cells are now ready for acquisition.

Notes: Exclusion of dead cells in FACS staining using 7-AAD (7-AAD: Sigma A9400-1MG)

General

7-Amino-actinomycin D (7-AAD) is a fluorescent chemical compound with a strong affinity for DNA. It is used as a fluorescent marker for DNA in fluorescence microscopy and flow cytometry. It intercalates in double-stranded DNA, with a high affinity for GC-rich regions.
With an absorption maximum at 546 nm, 7-AAD is efficiently excited using a 543 nm helium-neon laser; it can also be excited with somewhat lower efficiency using the 488 nm or 514 nm argon laser lines.
7-AAD does not readily pass through intact cell membranes; 7-AAD is used as a cell viability stain. Cells with compromised membranes will stain with 7-AAD, while live cells with intact cell membranes will remain dark.

Specific

On a FACSCalibur, 7-AAD staining is detected in the FL3 channel. Therefore you cannot use PerCp-conjugated antibodies for these samples (or other fluorophores that are detected in FL3).

DO NOT FIX YOUR SAMPLES!!!

The 7-AAD stock vial is located in the "FACS antibody stock box" in the FACS antibody fridge in 3N11B. The stock concentration is 1mg/ml. Final concentration for staining of dead cells will be 0.5-1µg/ml.
To make up a new stock vial, dissolve 1mg of 7-AAD in 50µl absolute methanol (3N42) and add 950µl PBS.
Open the 7-AAD vial ONLY in the sterile hood, because it is used for staining of samples for FACS sorting.

Procedure

As for every FACS staining, you need single stains for every color. Therefore you must include a single stain for 7-ADD for compensation.
Important: Make sure that your unstained sample and your single stains (except the 7-AAD single stain) DO NOT get treated with 7-ADD!

- After your last wash step, re-suspend cells in 400µl FACS buffer.
- Dilute 7-AAD 10x in FACS buffer. You need 0.4µl of stock 7-AAD per 400µl sample.
  E.g. if you have 30 samples, dilute 12µl 7-ADD (1mg/ml) in 108µl (=120-12) FACS buffer.
- Add 4µl of the diluted 7-ADD to your sample (400µl) and mix well.
- TIPP: To make pipetting easier, dilute 7-ADD again 10x in FACS buffer (e.g. add 1200-120=1080µl FACS buffer to the 120µl 7-ADD solution) and add 40µl per 400µl sample.
- If your sample is more than 400µl (e.g. for FACS sorting) adjust the amount of 7-AAD accordingly.
- Most protocols recommend staining for 5-20min. However, for simple dead cell exclusion, 7-AAD can be added just before acquiring the samples on the FACSCalibur.
- DO NOT add 7-AAD hours before acquiring!