Staining of PCNA as an S-phase marker for Flow Cyometry

Based on Sasaki et al, PNAS, 1993

Collect cells by trypsinization and wash with PBS. Count and take 1 million for the staining.

1. Spin 500g, 5 min, 4 °C
2. Resuspend in 100 uL PBS
3. Pre-extract by adding 500 uL PBS/0.1 % Triton X-100/1%BSA, and incubate 10 min on ice.
4. Add 3 mL methanol at -20 °C and incubate 3 min at -20 °C
5. Spin 500 g, 5 min
6. Resuspend in 1 mL PBS (pellet difficult to resuspend) and store at 4 °C until day of FACS (no later than next day)

DAY OF FACS

Solutions needed:

PI/TritonX-100 with RNase (Freshly made)

10 mL of 0.1% (v/v) TritonX-100 in PBS
2 mg DNase-free RNaseA (we use Thermo EN0531, stock is 10 mg/ml → 200 uL)
200 uL of 1mg/ml PI

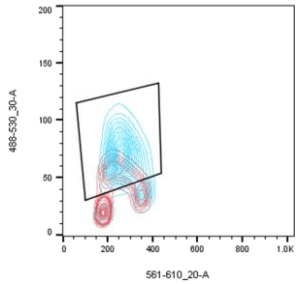
Rinsing buffer: 1% (w/v) bovine serum albumin (BSA) in PBS, pH 7.4 (store at 4°C)

Antibody used: PCNA (PC10) Mouse mAb (Alexa Fluor® 488 Conjugate), CST8580. Use 1:50

1. Centrifuge the cells 5 min at 1000xg. Remove the alcohol.
2. Resuspend cell pellet in 5 mL PBS, wait 15 min at RT
3. Centrifuge 5 min at 1000xg, RT
4. Block in 3 mL rinsing buffer for 10 min at RT
5. Spin 5 min at 1000xg, RT
6. Resuspend the pellet in 50 uL rinsing buffer with the labeled primary corresponding antibody.
7. Incubate 1h at RT with gentle agitation (in the dark)
8. Add 5 mL rinsing buffer, spin 5 min at 500xg RT
9. Suspend cell pellet in 300uL PI/TritonX-100 with RNaseA.
10. Incubate 30min @RT.

Example of HeLa cells untreated (red) or synchronized with a double thymidine block and released for 4 hours (blue)

Optimal staining (inverted V shape):



Frequently staining looks like this: