DAPI staining for cell cycle analysis

Specificity: Binds preferentially A-T base regions in DNA.

Process between 1 - 10 million of cells

1. Spin the cells at 500g, 5 min, 4°C.
2. Wash twice with 5mL cold PBS 1X, spin 5 min, 500 g, at 4°C.
3. Resuspend carefully the cell pellet in 500µL of PBS 1X (make sure to obtain a single-cell suspension).
4. Add the cell suspension dropwise to a 15mL Falcon tube containing 4.5 mL of ice-cold 70 % EtOH while vortexing slightly.
5. Incubate at 4°C for at least 2 hours.
   Cells can be stored in fixative at 4°C for several days, for longer storage keep them at -20).
6. Day of the staining: centrifuge the ethanol-suspended cells 5min at 1000g. Remove the supernatant carefully.
7. Resuspend the cell pellet in 5ml PBS 1X, wait 15 min at RT.
   During this time, count the cells again and distribute in tubes the same cell number (1-2 million cells)
8. Spin the cells 5min at 1000g.
9. Resuspend the cell pellet in 300µL of DAPI/TritonX-100.
10. Incubate 30 minutes at RT protected from light.
11. Transfer the sample to appropriate tubes for acquisition at the cytometer. Read DAPI signal in parameter: ***355-450/50***, lineal mode. Acquire your samples in **Low.**

DAPI/Triton X-100 Solution: To 10ml of 0.1% (v/v) TritonX-100 (prepared in PBS) add 10µL of 1mg/ml DAPI.

To Consider:

-Keep the same ratio DAPI:Number of cells

-Prepare always an asynchronous culture (untreated) to fix the positions of the G1 and G2 peaks.

References

Protocol slightly modified from: Current Protocols in Cytometry (1997) 7.5.1-7.5.24 Copyright © 1997 by John Wiley & Sons, Inc. Contributed by Zbigniew Darzynkiewicz and Gloria Juan.