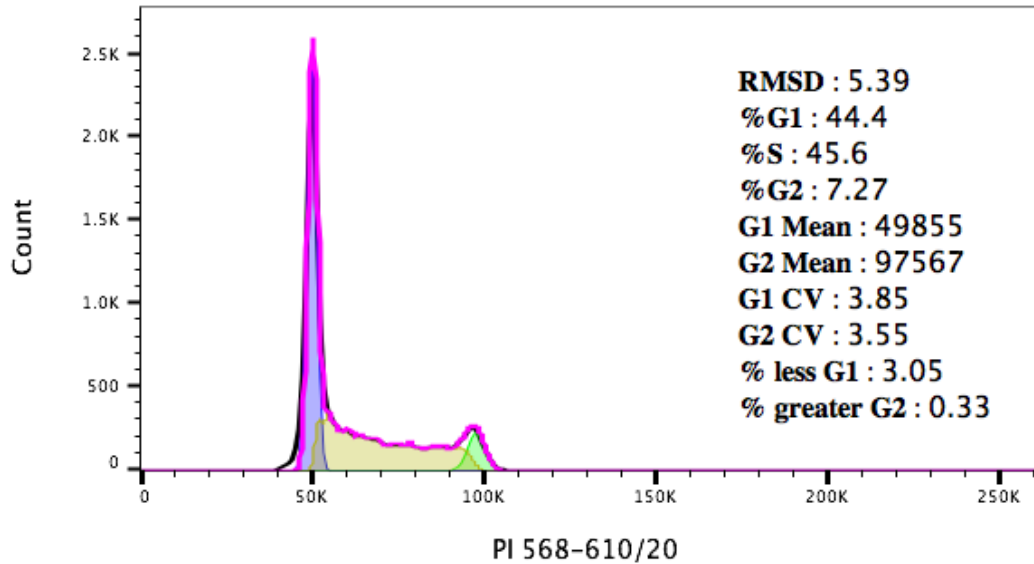


Propidium Iodide Staining

Specificity: Interbase-intercalating agent.



Data from Amparo Andres-Pons/Beck Group

Process between 1 - 10 million of cells

1. Spin the cells at 500g, 5 min, 4°C.
2. Wash twice with 5 mL cold PBS 1X, spin 5 min, 500 g, at 4°C.
3. Resuspend the cell pellet in 500µL of PBS 1X very carefully (make sure to obtain a single-cell suspension).
4. Add the cell suspension dropwise to a 15 mL 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 10min 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 PI/TritonX-100 + RNase.
10. Incubate 15min at 37°C or 30 minutes at RT protected from light.
11. Transfer the sample to appropriate tubes for acquisition at the cytometer. Read PI signal in parameter: **561-610/20**, lineal mode. Acquire your samples in **Low**.

PI/Triton X-100 + RNase Solution:

To 10ml of 0.1% (v/v) TritonX-100 (prepared in PBS) add:

200µL of 1mg/ml PI stock

2mg DNase-free RNase A (stock from Thermo is 10 mg/ml → 200 µL)

To Consider

- Keep the same ratio PI:Number of cells
- Prepare always an asynchronous culture (untreated) to fix the positions of the G1 and G2 peak.

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. Modified and kindly donated by Amparo Andres-Pons/Beck Group.