Cell Cycle Determination Using DAPI – Alcohol Fixation Method

DAPI is commonly used in cell cycle analyses since it preferentially binds dsDNA, when cells are permeabilized, allowing it to intercalate with and saturate the nucleic acids. DAPI will also bind to dsRNA, but gives emits at a longer wavelength near 500 nm.

The protocol below may be used for DAPI measurements alone and should not be combined with other dyes or surface markers since alcohol based fixatives degrade the cellular membrane as well as most fluorescence based probes.

Required: A flow cytometer with a UV (355 nm, 375 nm) or Violet (405 nm) laser is required for DAPI analysis with detectors at 450 nm

Preparation of DAPI working solution:

- DAPI: Sigma D9542 (10 mg)
- DAPI Stock solution: Dissolve 1mg of DAPI in 1 mL in water (can be stored for several months in the dark in foil wrapped tubes at -20°C. Aliquot into 15 µL Aliquots.
- DAPI Working solution (10 µg/mL): To 15 mL 0.1% Triton X – 100 in PBS solution, add 15 µL of DAPI stock solution (prepare fresh each time).

Method:

1. Prepare cell suspensions in the normal manner for the cell line/type of interest.
2. Adjust concentrations to 1 x 10^6 cells/mL.
    a. Centrifuge cells for 10 min at 1000 rpm
    b. Aspirate supernatant using a vacuum
3. Suspend cells in 50 µL of HBSS containing 2% FBS (pipette up and down a few times if your cells readily form aggregates).
    a. Add 1mL of ICE cold 70% ethanol in a dropwise manner while mixing gently on a vortex (minimizes new aggregate formation)
    b. Store on ice for no less than 2 hours
    c. This is a good place to stop if you intend to stain the cells at another time. Cells can be stored in ethanol for several weeks to months.
4. Pellet cells and carefully remove supernatant (fixed cells will not sediment as tightly as live cells, take care not to aspirate to dryness or disrupt the pellet and thereby lose cells).
    a. Wash 2X with HBSS + 2% FBS
5. Add 1mL DAPI working solution
6. Incubate in the dark for 15 - 30 minutes at RT
7. Filter through 40 µM mesh filter
8. Do not wash.

Method Notes:

Proper DNA saturation is the key to good cell cycle analysis and should not vary between samples. The variance comes from too much/too little DNA per amount of DNA intercalating/binding dye added to the sample resulting in shifting DNA content peaks i.e. G1 will not appear at the same place on the scale for each sample.

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This method may work with some intracellular markers, but alcohol fixation tends to destroy most surface markers. Paraformaldehyde fixation is recommended if multiplexing cell cycle analysis with other markers is required.

References:

1) Invitrogen.com:
https://catalog.invitrogen.com/index.cfm?fuseaction=viewCatalog.viewProductDetails&productDescription=3817

2) Tarnowski BI, Sens DA, Nicholson JH, Hazen-Martin DJ, Garvin AJ, Sens MA.,Automatic quantitation of cell growth and determination of mitotic index using DAPI nuclear staining., Pediatr Pathol. 1993 Mar-Apr;13(2):249-65.

3) Tarnowski BI, Spinale FG, Nicholson JH., DAPI as a useful stain for nuclear quantitation.,Biotech Histochem. 1991;66(6):297-302.

4) Otto F., DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA., Methods Cell Biol. 1990;33:105-10.

5) Darzynkiewicz, Z. and Juan, G. 2001. DNA Content Measurement for DNA Ploidy and Cell Cycle Analysis. Current Protocols in Cytometry. 00:7.5:7.5.1–7.5.24.