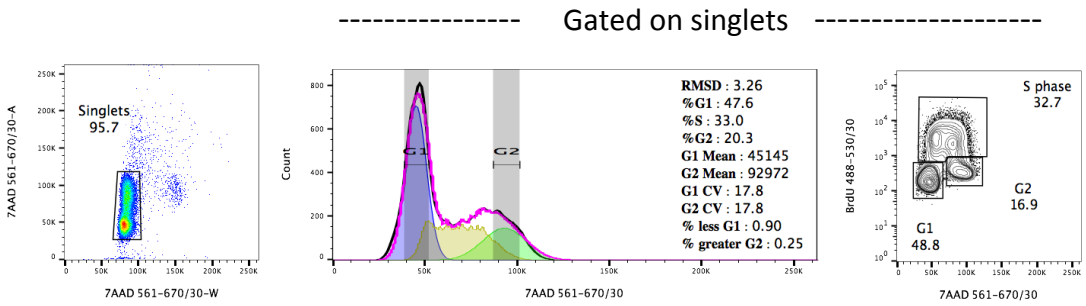
BrdU Staining

The immunofluorescent staining of incorporated bromodeoxyuridine (BrdU) and flow cytometric analysis provide a high resolution technique to determine the frequency and nature of individual cells that have synthesized DNA. In this method, BrdU (an analog of the DNA precursor thymidine) is incorporated into newly synthesized DNA by cells entering and progressing through the S (DNA synthesis) phase of the cell cycle. The incorporated BrdU is stained with specific anti-BrdU fluorescent antibodies. The levels of cell-associated BrdU are then measured by flow cytometry.

Often, staining with a dye that binds to total DNA such as 7-amino-actinomycin D (7-AAD) is coupled with immunofluorescent BrdU staining. With this combination, two-color flow cytometric analysis permits the enumeration and characterization of cells that are actively synthesizing DNA (BrdU incorporation) in terms of their cell cycle position (ie, G0/1, S, or G2/M phases defined by 7-AAD staining intensities).



Data from Elisabeth Zielonka/Hentze Group

Protocol:
All centrifugations steps: 1400 rpm at 4°C.

1. Staining of cell surface antigens.

a. Add BrdU-pulsed cells (1x106) to flow cytometry tubes spin and discard supernatant.
b. Add 100ul of ab mix for cell-surface markers diluted in staining buffer.
c. Incubate cells for 20 minutes on ice protected from light.
d. Wash cells with 1ml of staining buffer per tube, spin and discard supernatant.

2. Fix and permeabilize cells with BD Cytofix/Cytoperm Buffer.
a. Resuspend cells in 100µl of BD Cytofix/Cytoperm Buffer per tube.
b. Incubate cells for 15 – 30 minutes at room temperature or on ice.
c. Wash cells with 1ml of BD Perm/Wash Buffer 1X, spin and discard supernatant.

3. Incubate cells with BD Cytoperm Plus Buffer.
a. Resuspend cells in 100µl of BD Cytoperm Plus Buffer per tube.
b. Incubate cells for 10 minutes on ice.
c. Wash cells with 1ml of BD Perm/Wash Buffer 1X, spin and discard supernatant.

4. Re-Fixation of cells
a. Resuspend cells in 100µl of BD Cytofix/Cytoperm Buffer per tube.
b. Incubate cells for 5 minutes at room temperature or on ice.
c. Wash cells with 1ml of BD Perm/Wash Buffer 1X, spin and discard supernatant.

5. Treatment of cells with DNase to expose incorporated BrdU.
a. Resuspend cells in 100µl of diluted DNase
b. Incubate cells for 1 hour at 37°C.
c. Wash cells with 1ml of BD Perm/Wash Buffer 1X, spin and discard supernatant.

6. Stain BrdU and intracellular antigens with fluorescent antibodies.
a. Resuspend cells in 50µl of BD Perm/Wash Buffer containing diluted fluorescent anti-BrdU and/or antibodies specific for intracellular antigens.
b. Incubate cells for 20 minutes at room temperature.
c. Wash cells with 1ml of BD Perm/Wash Buffer 1X, spin and discard supernatant.

7. Optional — Staining of total DNA for cell cycle analysis.
Note: Proceed to Step 8 if the staining of total DNA levels is not desired.
a. Resuspend cells in 20µl of the 7-AAD solution.
8. Resuspension of cells for Flow Cytometric Analysis.
a. Add 1 ml of staining buffer to each tube to resuspend cells.
b. Analyze stained cells with a flow cytometer (run at a rate no greater than 400 events/sec.).

Note: Samples may be stored overnight at 4°C, protected from exposure to light, prior to analysis by flow cytometry.

Reagents

BD Perm/Wash Buffer 10X: The concentrated stock buffer should be diluted 1:10 with deionized H2O. The presence of some precipitate in the 10× BD Perm/Wash stock buffer is common. The precipitate will not affect the performance of the buffer. If desired, the precipitate may be removed prior to use by filtration of the diluted 1× BD Perm/Wash Buffer through a 0.45 µm-pore filter.

DNase solution: Each vial contains 300µl of a 1 mg/ml solution of DNase in 1× DPBS. When staining 10 or more samples, thaw the entire vial of DNase solution and add 700µl of 1× DPBS to make a working stock solution of 300 µg/ml. A total of 100µl of the working stock is used to treat each cell sample.

Anti-BrdU Antibody: Dilute 1:50 in BD Perm/Wash Buffer 1X. Use 50µl of diluted antibody per sample.

References

BrdU Flow Kits, Instruction Manual. BD Pharmingen™