DNA Staining Protocol for Flow Cytometry

Materials and Reagents

Full Name	Short Name	Catalog Number
PBS		
BD Cytofix™ fixation buffer (optional, for alternative fixation step)	Fixation Buffer	554655
Ethanol, 70–80%, stored at -20°C		
BD Pharmingen™ stain buffer, or equivalent, 1X PBS, 2% FBS, 0.1% NaN_3 (pH 7.1–7.4)	Stain Buffer	554656
BD Pharmingen™ PI/RNase staining buffer (for PI/RNase staining)	PI/RNase Staining Buffer	550825
BD Pharmingen™ 7-AAD (7-Amino-Actinomycin D) staining solution (for 7-AAD staining)	7-AAD Staining Solution	559925

Procedural Note

- DNA dyes, especially propidium iodide (PI), can be sticky. After running the samples, clean the flow cytometer before the next use according the instructions provided in the cytometer User's Guide.
- Fixed cells can be used up to 12 months after fixing (stored in 70 to 80% ethanol at -20°C).

Procedure

1. Pellet the cells and wash them by adding 30 to 40 mL of PBS.
2. Centrifuge the cells at 1,000 rpm for 10 minutes and aspirate the supernatant.
3. [Optional] Fix the cells with Fixation Buffer for 15 to 30 min at 4°C.
4. While vortexing (to loosen the pellet), add 5 mL of cold 70 to 80% ethanol drop by drop into the cell pellet. Incubate at -20°C for 2 hours minimum.
5. Wash twice to remove the ethanol. Perform the first wash in 1X PBS and the second wash in Stain Buffer.
6. Centrifuge the cells for 10 minutes at 1,000 to 1,500 rpm and aspirate the supernatant.
7. To stain, use 10^6 cells per test and do one of the following:
   - For PI/RNase staining, resuspend the cells in 0.5 mL of PI/RNase Staining Buffer.
   - For 7-AAD staining, resuspend the cells in 0.1 mL of Stain Buffer and add 20 µL of 7-AAD Staining Solution.
8. Incubate 15 minutes at room temperature.
9. Store tubes at 4°C protected from light prior to analyzing. Analyze on the flow cytometer within 1 hour.