Protocol to Determine Mitotic Index by FACS

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[Abstract] Fluorescence Activated Cell Sorting (FACS) is a sensitive method to count mitotic cells. Cells are stained with an antibody that recognizes an antigen present only in mitotic cells, combined with propidium iodide (PI) to stain DNA. Two-dimensional FACS scanning allows the differential quantitation of G2 and mitotic cells. Several antibodies to different mitotic markers have been used in the community, including antibodies to MPM-2 antigens present in mitotic cells. MPM-2 recognizes a phosphorylated epitope (LTPLK or YWFSPL) 6, 7 in a distinct class of phosphoproteins including MAP2, HSP70, cdc25, and DNA topoisomerase IIα, most of which are phosphorylated at the onset of mitosis. The commercial availability and specificity of antibodies to histone H3 phosphorylated at threonine 11, which is present only in mitotic cells, has also been widely used to detect mitosis cells.

Materials and Reagents

1. Phosphate buffered saline (PBS)
2. Ethanol
3. Triton X-100 (Sigma-Aldrich, catalog number: T9284)
4. BSA (Sigma-Aldrich, catalog number: A3803)
5. MPM-2 monoclonal antibody
6. Anti-phospho-Histone H3 (Thr11) (EMD Millipore, catalog number: 06-570)
7. Anti-phospho-Ser/Thr-Pro, MPM-2 (EMD Millipore, catalog number: 05-368)
8. Alexa 488-conjugated goat anti-mouse immunoglobulin G antibody (Life Technologies, Molecular Probes®/Alexa Fluor® 488, catalog number: A-11008 or A-11034)
9. RNase A (Sigma-Aldrich, catalog number: R4642)
10. PI (Sigma-Aldrich, catalog number: P-4170)

Equipment

1. Centrifuges (Beckman Falcon, TLS-55)
2. Incubator
3. FACS machine

4. FACS tubes (BD Biosciences, Falcon®, catalog number: 352054)

Procedure

Note: All spins are done at 2,000 rpm for 5 min.

1. After collecting the cells (of your choice), wash them with 500 µl of PBS once, resuspend in 150 µl of PBS and then add 350 µl ethanol. Mix and store cells at 4 °C for at least 1 h.
2. Spin and remove ethanol. Resuspend cells in 500 µl of PBS containing 0.25% Triton X-100 and incubate on ice for 15 min.
3. After centrifugeation, the cell pellet was suspended in 100 µl of PBS containing 1% BSA and 0.25 µg of Histone H3 monoclonal antibody or 0.06 µg MPM-2 monoclonal antibody and incubated for 1 h at room temperature (RT).
   Note: After this step, cell pellets become loose even after centrifugation, therefore it is better to use a pipet to remove the solutions rather than using an aspirator.
4. Spin and wash with 150 µl of PBS containing 1% BSA once.
5. Resuspend cells in Alexa 488-conjugated goat anti-mouse immuneoglobulin G antibody diluted at a ratio of 1:300 in 100 µl of PBS containing 1% BSA and incubate at RT in the dark for 30 min.
6. After centrifugation, resuspend cells in 500 µl of PBS containing 10 µg/ml RNase A and 20 µg/ml PI, transfer to FACS tubes and incubate at RT in the dark for 30 min.
7. Take sample to FACS immediately or store at 4 °C until FACS analysis.

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