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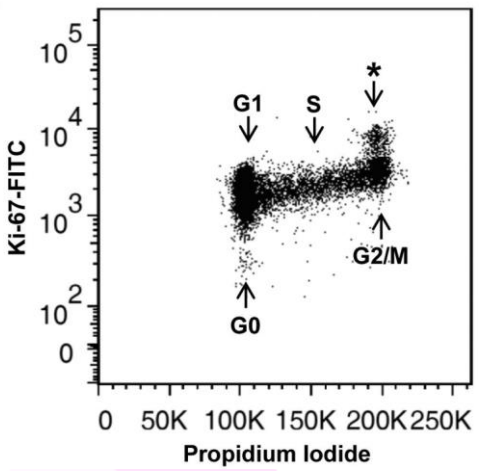
Ki-67 ANALYSIS BY FLOW CITOMETRY

- Analyze both proliferation specific marker (Ki-67) and cellular DNA content, which discriminates resting/quiescent cell populations (G0 cell) and quantifies cell cycle distribution (G1, S or G2/M, respectively).
- Set up and adjust flow cytometer and detection filters. Acquire Ki-67 signal in logarithmic mode and exclusion non viable cells dye signal in linear mode.
- Set a low flow rate (less than 400 events/second) for optimal resolution of exclusion non viable cells dye fluorescence. Exclude doublets
- Acquire the fluorescence and analyze the cell cycle stages of each sample

Kinetics of cell cycle status can be assessed by cell proliferation assays based on the measurement of newly synthesized DNA content and cell metabolism parameters.
Based on differences in Ki-67 expression level (Figure 1), the resting/quiescent (G0) population can be discriminated from other proliferating cells (G1, S, G2/M Phases).
Generally, G0 cells have lower Ki-67 levels, so these cells can be distinguished from proliferating cells.
To accurately quantify the cell cycle distribution of proliferating cells, different cell cycle analysis software are used.
Previous studies have demonstrated that a small portion of cells showed a significant increase of Ki-67 level in G2/M cells (Figure 1A, asterisk). These cells were regarded as early mitotic cells. To further separate M phase cells, other markers such as Cyclins, MPM-2 and phospho-Ser10-histone H3 (to detect M phase) need to be combined.



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