

Labeling of Nuclei with BrdU and PI for FACS

This protocol uses acid treatment to denature the DNA. Other protocols use thermal denaturation, which is more efficient and therefore more sensitive for BrdU incorporation. However, thermal denaturation results in greater cell loss. The following protocol works well for Rat 1a cells.

1. Fix cells in 70% EtOH as for standard PI staining.
2. Centrifuge at 3.5K in microfuge for 10min at 41/4°C. Discard supernatant.
3. Resuspend pellet and add 200 ul of 2M HCl/Triton -100 (this will cause aggregation of the cells). Incubate at room temperature for 30mins to denature the DNA. Mix gently every 10min-tap a little.
4. Centrifuge at 3.5K in microfuge for 10 min at room temperature.
5. Aspirate off supernatant, resuspend pellet and add 200 ul of 0.1M Na₂B₄O₇, pH 8.5 to neutralize the acid.
6. Count the cells and transfer ~1x10⁵ to an eppendorf tube. If the pellets look similar, just count 1 or 2.
7. Centrifuge cells at 12,000G for 10 sec and aspirate off supernatant.
8. Resuspend cells in 20 ul of PBS + 0.5% Tween 20 + 1% BSA and add 10 ul of anti-BrdU FITC (Becton Dickinson, Cat. No. 7583) per 1x10⁶ cells. Incubate for 30 min at room temperature in the dark.
9. Spin down cells at 3.5K in microcentrifuge for 10 min, aspirate off supernatant and wash in 50 ul of PBS + 0.5% Tween 20 + 1% BSA.
10. Resuspend cells in 200 ul 38 mM NaCitrate + 69 uM propidium iodide. Add 1 ul of 9.5 mg/ml RNaseA (19 ug/ml final). Incubate for 30 min at 37 1/4°C in the dark.

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