

2-Color Cell Cycle With BrdU/PI Protocol

Reagents

PBS, Ca ⁺⁺ , Mg ⁺⁺ -free	1% BSA/PBS	
2N HCL/0.5% Triton X-100	70% Ethanol	
Normal Saline (0.9% NaCl)	0.5% Tween-20/1% BSA/PBS	
Propidium Iodide (PI) 1mg/ml stock:	[PI] _F = 5ug/ml	PI Master Mix
1mM BromodeoxyUridine (BrdU)	PBS	995 ul
Goat F(ab') ₂ anti-Mouse IgG-FITC	PI	5 ul
0.1M Sodium Tetraborate (Na ₂ B ₄ O ₇ 10H ₂ O), pH 8.5		1,000 ul
anti-BromodeoxyUridine monoclonal antibody (α-BrdU mAb)		

Procedure

1. Cells under optimum growing conditions (log growth phase at critical cell density). Add BrdU at a final concentration of 10uM. Incubate for 30-60 minutes. (Time of pulse and BrdU concentration variable with cell type and doubling time*. Ranges from 15' to 4 hours, and from 10uM to 100uM)
2. Wash cells 2X in 1% BSA/PBS. Re-suspend cells in 100ul normal saline.
3. Place 5mls 70% Ethanol (ice cold) in a glass tube and vortex. Slowly add the 100ul cells/saline while vortexing. Incubate 45' on ice.
4. Centrifuge cells (~1,500 rpm, 10', 4⁰C to avoid cell loss). Aspirate supernatant completely.
5. Add 1ml 2N HCl/0.5% Triton X-100 and incubate 30' at room temp.
6. Pellet cells and remove supernatant. Re-suspend cells in 1ml 0.1M Sodium Tetraborate. Pellet cells.
7. Re-suspend cells in 50ul 0.5% Tween-20/1% BSA/PBS. Add 10-20ul (1ug/10⁶ cells) α-BrdU (mAb) and incubate for 45' at room temp.
8. Pellet cells and re-suspend in 50ul 0.5% Tween-20/1% BSA/PBS. Add 5ul (1ug/10⁶ cells) Goat F(ab')₂ anti-Mouse IgG-FITC. Incubate 30' at room temp.
9. Pellet cells and re-suspend in PI master mix. Provide the proper controls and a PI wash tube approx. 1ml for each 5 samples.

Time (hours) X Ln2

* Doubling Time = τ_D

$$\tau_D \text{ (Hr.s)} = \frac{\text{Time (hours)} \times \text{Ln}2}{\text{Ln} \frac{N_f}{N_i}}$$

Nf = final cell count

Ni = initial cell count