

BrdU Incorporation

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Grow cells to an exponential phase ($5-10 \times 10^5$ /ml).

Add BrdU (stock; 10mM) to culture medium at 20 μ M.

Incubate for 20 min.

Harvest cells (10^6 cells) by centrifugation at 1,200rpm for 3min.

Resuspend cells in 50 μ l of PBS very well (important).

Add 10 ml of 70 % ethanol at -20°C .

(Cells can be stored at -20°C up to 1 month.)

Centrifuge at 1,700rpm for 3 min.

Resuspend in 1ml of 1% BSA-PBS.

Centrifuge at 1,700rpm for 3 min.

Resuspend in 1 ml of 4M HCl-0.5% TritonX-100. Mix well by pipetting.

Incubate for 30 min at room temperature.

Centrifuge at 1,700rpm for 3 min.

Resuspend in 5ml of 1% BSA-PBS.

Centrifuge at 1,700rpm for 3 min.

Resuspend in 5ml of 1% BSA-PBS.

Centrifuge at 1,700rpm for 3 min. (check pH of supernatant. Should be 7).

Add 30 μ l of anti-BrdU (BectonDickinson, Cat# 347580). Mix well.

Incubate for 60 min at room temperature.

Add 1 ml of 1% BSA-PBS and centrifuge at 1700rpm for 3 min.

Resuspend in 1ml of 1% BSA-PBS.

Centrifuge at 1,700rpm for 3 min.

Add 30 μ l of anti-mouse IgG-FITC (Jackson ImmunoResearch Labs, Inc, Cat #318-095-003, 1/20: diluted with 1% BSA-PBS.)

Incubate for 30 min at room temperature in dark.

Add 1 ml of 1% BSA-PBS and centrifuge at 1,700rpm for 3 min.

Resuspend in 500 μ l of 1% BSA-PBS (PI: 5 μ g/ml)

Incubate for 1h (more).

Filter cells and perform FACS analysis (FL1 log, FITC vs FL2 linear, PI).