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NB100-64345 Protocol

FLOW CYTOMETRY (NB100-64345)

Prepare the following solutions before proceeding:
Phosphate buffered saline (PBS)
2N HCl containing 0.5% Triton X-100
PBS containing 0.05% Tween-20
PBS containing 1% BSA (PBS/BSA)
10mg/ml Propidium iodide (PI)
0.1M $Na_2B_4O_7$, pH 8.5

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 umol/L and incubate for 30 minutes in a CO_2 incubator at 37 degrees C.
2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 minutes, decant supernatant and resuspend in a minimum volume of PBS.
3. Add cells slowly into 5ml of 70% ethanol at -20 degrees C, mixing continuously (vortex preferred). Incubate on ice for 30 minutes.
4. Centrifuge at 500g for 10 minutes, decant supernatant, and resuspend cell pellet.
5. Add 2ml of 2N HCl containing 0.5% Triton X-100 and incubate the cells for 30 minutes at room temperature (preferably on a rocking platform).
6. Centrifuge at 500g for 10 minutes, decant supernatant and resuspend in 3 ml of 0.1M $Na_2B_4O_7$, pH 8.5
7. Centrifuge at 500g for 10 minutes, decant supernatant and resuspend the cells in PBS/BSA + 0.05% Tween-20. Adjust cell concentration to 1 x 107/ml.
8. Aliquot 100ul of cell suspension into required number of 12 x 75mm tubes.
9. Incubate the cells with the BrdU antibody at the recommended dilution for 45 minutes at room temperature or overnight at 4 degrees C.
10. Add 2 ml of PBS/BSA and centrifuge the cells at 1000rpm for 5 minutes.
11. If a secondary antibody layer is required then decant the supernatant and incubate the cells with the secondary antibody for 30 minutes at room temperature. If no secondary antibody layer is required then proceed to step 13.
12. Wash the cells after the secondary antibody layer by repeating step 10.
13. Decant the supernatant and add 1ml of PBS containing 10ug/ml PI (Dilute the 10mg/ml solution of PI 1/1000 in a suitable volume of PBS).
14. Analyze cells by flow cytometry following the manufacturer's instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale