Cell Cycle Analysis

Using Propidium Iodide & Bromodeoxyuridine

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REAGENTS NEEDED:

* Dulbecco's PBS
* 10mM BrdU Stock Solution: Dissolve 30mg in 10mL of culture media. Store in refrigerator in dark container (foil-wrapped). KEEP BrdU SOLUTION IN THE DARK. VERY LIGHT SENSITIVE!!!
* 4N HCl Solution: Dilute 10mL of concentrated HCl (12N) with 30mL of H2O.
* 0.5% Tween 20 Solution: Dilute 100μL of Tween 20 with 19.9mL of Dulbecco's PBS.
* Propidium Iodide Stock Solution: Dissolve 10mg of P.I. in 100mL of Dulbecco's PBS. Store in refrigerator for up to 1 month or store frozen (1mL aliqots) for up to 1 year.

PROCEDURE

(This procedure requires 3 to 4 hours and must be done in minimal light.)

1. Suspend cells in culture medium at a concentration between 0.5 x 106 and 1 x 106 (½ - 1 million) cells per mL.
2. Add 20μL of BrdU stock solution per each mL of cells. KEEP THE CELLS IN THE DARK FROM THIS POINT ON.
3. Incubate cells for 30 min. in 37°C incubator
4. Transfer cells to centrifuge tube.
5. Centrifuge cells and dump culture media supernatant.
6. Wash cells twice with cold PBS.
7. Centrifuge cells and dump the PBS supernatant (Do not blot or aspirate all of the supernatant.).
8. Resuspend the cell pellet in the residual supernatant. If cells are not resuspended, they will clump when the ethanol is added.
9. Fill tube with cold 70% ethanol and incubate cells for 30 min. on ice. (The fixed cells are now stable and can be stored in the dark (foil-wrapped) in the refrigerator and the remainder of the procedure can be completed later.)
10. Centrifuge cells and dump the ethanol supernatant.
11. Resuspend cell pellet in the residual supernatant.
12. Fill the tube with 4N HCl and incubate at room temperature for 30 min.

13. Centrifuge cells and dump HCl supernatant.
14. Resuspend cells in 1mL of PBS for each 106 cells.
15. Aliquot 1mL of cells into each 12x75 polystyrene (Falcon) tube.
16. Centrifuge cells and dump PBS supernatant.
17. Wash cells once with 0.5% Tween 20 solution.
18. Centrifuge cells and dump Tween 20 supernatant.
19. Add 10μL of B-D FITC-conjugated anti-BrdU antibody to each tube, mix by vortexing and incubate at room temperature for 30 min.
20. Wash cells twice with PBS.
21. Add 1mL of PBS and 10μL of PI solution to each tube.
22. Analyze by flow cytometry or store in the dark (foil-wrapped) in refrigerator until analyzed. (Stable for up to one year.)

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* To return to the list of methods.
* To return to the Cooper Cytometry homepage.