Mouse Cell Cycle Experiments.

- ✧ Experimental preparation
  1. BD Cycletest™ Plus DNA Kit
     i. Buffer Solution (room temperature).
     ii. Solution A (room temperature).
     iii. Solution B (room temperature).
     iv. Solution C (on ice, without light, PI).
  2. Cell culture preparation
     Cell amount: $3x10^5$ cells/sample.
- ✧ Experimental procedure
  1. Place cells from culture plate into tube.
  2. Centrifuge the cell suspensions at 1500 rpm for 5 minutes at room temperature (20°C–25°C).
  3. Aspirate the supernatant, leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.
  4. Add 1 mL of Buffer Solution and resuspend the cells by gently vortexing at low speed.
  5. Centrifuge for 5 minutes at 1500 rpm at room temperature (20°C–25°C).
  6. Repeat step 3 through step 5.
  7. Aspirate the supernatant, leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet.
  8. Resuspend the pellet in 1 mL of Buffer Solution by gently vortexing at low speed.
  9. Count the cells.
  10. Adjust the concentration to 1.0×10^6 cells/mL with Buffer Solution. ($3x10^5$ cells in the 300 µL Buffer Solution) This should provide sufficient cells for both a test sample tube and a control tube.
  11. Centrifuge the cell suspensions at 1500 rpm for 5 minutes at room temperature (20°C–25°C).
  12. Carefully decant all the supernatant.
  13. Add 250 µL of Solution A (trypsin buffer) to each tube and gently mix by tapping the tube by hand. ***Do not vortex.***
  14. Incubate for 10 minutes at room temperature (20°C– 25°C). ***Do not aspirate Solution A.***
  15. Add 200 µL of Solution B (trypsin inhibitor and RNase buffer) to each tube and gently mix by tapping the tube by hand. ***Do not vortex.***
  16. Incubate for 10 minutes at room temperature (20°C– 25°C). ***Do not aspirate Solution A and B.***
  17. Add 200 µL of cold (2°C–8°C) Solution C (PI stain solution) to each tube and gently mix by tapping the tube by hand. ***Do not vortex.***
  18. Incubate for 10 minutes in the dark on ice or in the refrigerator (2°C–8°C).
  19. Filter the sample into a tube.

  The samples are now ready to be analyzed on the flow cytometer. Cap or cover the prepared tubes and store at 2°C– 8°C in the dark until flow cytometric analysis.