Cell Cycle / Apoptosis Analysis Kit

Cell Cycle / Apoptosis Analysis Kit can be used to measure Cell Cycle / Apoptosis activity in cells by flow cytometry.

Catalog number: ARG81299

Package: 200 tests

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INTRODUCTION

The content of DNA is changed with the process of cell cycle. And the DNA can be stained by fluorescent dye to measure its intensity by flow cytometry to monitor the cell cycle distribution in G1, S, GS/M phase and as well as apoptosis and aneuploidy cells with signals at sub-G1 or super G2 region.

Propidium iodide (or PI) is a fluorescent intercalating agent and it binds to DNA by intercalating between the bases with little or no sequence preference. Therefore PI is used widely as a DNA dye in flow cytometry to evaluate cell viability or DNA content in cell cycle analysis, or in microscopy to visualize the nucleus and other DNA-containing organelles. However, PI also binds to RNA, and it interference with the DNA measurement in the assay. In this Cell Cycle / Apoptosis Analysis Kit we provide an RNase to degrade RNA before PI staining to eliminate the interference by RNA and the kit can be used to monitor cell cycle progression, proliferation and apoptosis by flow cytometry.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped with blue ice. Store RNase A at-20°C upon receiving. Store other components at 2-8°C and protected from light. Use the kit before expiration date.

Component	Quantity	Storage information
Staining Buffer	4 X 20 ml (ready to use)	4°C
25 X Propidium Iodide (PI)	2 X 1.5 ml (ready to use)	4°C (Protect from light)
RNase A (2.5 mg/ml)	0.8 ml (ready to use)	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes and pipette tips
- Deionized or distilled water
- 95% ethanol
- 1X PBS: Add 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 to 800ml distilled water and adjust pH to 7.2-7.4. Finally, adjust the total volume to 1L. 0.2 μm filtered.
- Centrifuge
- Flow Cytometer (excited at 488/535 nm with a broad emission around 617 nm)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- RNase A should be stored at -20°C upon received. Store the other components in the kit at 4°C, protect from light.
- All reagents should be mixed well before using.
- Briefly spin down the reagents before use.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

- PI Working Reagent:

  For each reaction combine the following (*Prepare before use)*:

  - 0.4 ml of Staining Buffer
  - 15 µL of 25 X Propidium Iodide concentrate
  - 4 µL of RNase A (2.5mg/ml)

  It is recommended prepare freshly before use and prepare bulk working reagent using for once. Transfer 400 µl of PI Working Reagent to each sample.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

Standards and samples should be assayed in at least duplicates.

1. Cell preparation: Induce cells into apoptosis using proper method and including a mock-treated sample as a negative control.
2. Harvest cells:
   a. For suspension cells - Harvest by centrifugation at 1000 rpm for 5 minutes.
   b. For adherent cells
      a) Collect the cultured media including death cells.
      b) Rinse the attached cells with ice-cold PBS and collect the ice-cold PBS also.
      c) Detached cells with trypsin (with or without EDTA, however, EDTA free trypsin is recommended to avoid EDTA induced apoptosis).
      d) Harvest the cells with media.

e) Combine all collected media, PBS and cells from a), b) and d). Centrifuge at 1000 rpm for 5 minutes.

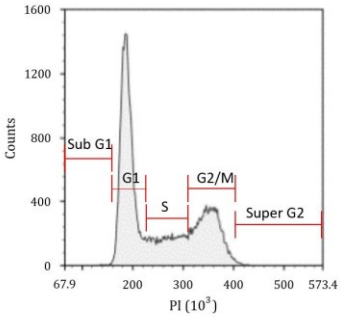
3. Wash cells: Remove the supernatant, wash the pallet with ice-cold PBS twice. After last centrifuge, discard the washing buffer (ice-cold PBS).
4. Fixation: Resuspend the cells with 1 ml of ice-cold PBS, and then slowly (drop by drop) add the cell resuspension in to 4 ml of 95% ice-cold ethanol with gently vertex. Incubate the cells on ice for at least 2 hours, or for overnight (12-24 hours is suggested).
5. Centrifuge samples at 1000 rpm for 5 minutes at 4°C, discard the supernatant.
6. Wash cells: Wash the pallet with 5 ml of ice-cold PBS. After Centrifuge, discard the washing buffer (ice-cold PBS).
7. Staining: Resuspend the cells with 400 µl of PI working solution (per sample), incubate at 37°C for 30 min in dark.
8. Wash cells: Wash the cells with 2 ml of ice-cold PBS. After Centrifuge at 1500 rpm for 5min, discard the washing buffer (ice-cold PBS).
9. Resuspend the cells with 400-500 µl of ice-cold PBS.
10. Samples are now ready to be analyzed. Analyze samples on a flow cytometer. Use 488 nm excitation line (Argon-ion laser or solid state laser) and emission collected at around 617 nm (orange, PI).

Note:

If the cells are not precipitated completely after each centrifuge step, the samples could centrifuge at 2000 rpm for 5-10 minutes to prevent lost cells.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only.



Sub G1	G1	S	G2/M	super G2
0.12%	40.85%	41.26%	17.75%	0.02%