Cell Cycle Assay Kit

User Manual

Catalog # CAK2006

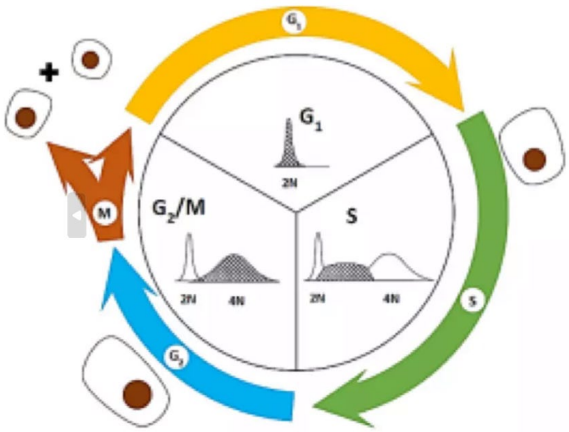
(Version 2.2B)

Detection cell cycle using flow cytometry.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Cell cycle detection is based on the property that propidium iodide (PI) specifically intercalates into the base pairs of double-stranded nucleic acids, with its fluorescence intensity being proportional to the intracellular DNA content. Since cells in G0/G1, S, and G2/M phases possess DNA contents of 2N, 2N-4N, and 4N respectively, flow cytometry can distinguish these subpopulations by detecting PI fluorescence intensity. RNase A is added prior to staining to degrade RNA, preventing non-specific binding of PI to RNA which would interfere with DNA quantification, thereby ensuring that the resulting histogram accurately reflects the cell cycle distribution.



II. KIT COMPONENTS

Component	50 Assays	100 Assays	Storage
Propidium Iodide (25X)	0.8 ml	1.6 ml	4 °C, Keep in dark
Staining Buffer	20 ml	20 ml x 2	4 °C
RNase A (5 mg/ml)	0.2 ml	0.4 ml	-20 °C
Manual	1	1	--

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. 5 ml and 10 ml graduated pipettes
2. 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
3. Beakers, flasks, cylinders necessary for preparation of reagents
4. Ice-cold 95% ethanol
5. Bench top centrifuge
6. Flow Cytometer
7. Ice-cold PBS

IV. ASSAY PROCEDURE

1. Upon reaching ~80% confluence, harvest adherent cells by trypsinization or collect suspension cells by centrifugation.
2. Wash cells with ice-cold PBS and centrifugate cells at 300~400 x g for 5 minutes.
3. Remove the supernatant and resuspend cells with ice-cold PBS and adjust cell concentration to 2 x 10^5 - 2 x 10^6/ml.
4. Slowly add 1 mL of cell suspension dropwise to 4 mL of ice-cold 95% ethanol while gently vertexing. Fix cells at 4 °C for 2 hours or overnight for 12~24 hours.
5. Centrifugate cells at 300~400 x g for 5 minutes. Extend the centrifugation time or increase the force slightly if pelleting is incomplete.
6. Remove the supernatant carefully and wash cells with 5 mL ice-cold PBS and centrifugate cells at 300~400 x g for 5 minutes. Remove the supernatant carefully and leave ~50 µL PBS. Gently flick the tube bottom to disperse the cell pellet adequately.
7. Staining Working Solution preparation: Prepare for immediate use. Store the unused solution in dark at 4°C for at most 24 hours. The volumes of reagent could be enlarged according to the specified numbers of well. The table below gives some examples.

No. of wells	Staining Buffer (ml)	Propidium Iodide (25X) (µl)	RNase A (5 mg/ml) (µl)
1	0.4	16	4
5	2	80	20
10	4	160	40
50	20	800	200
100	40	1600	400

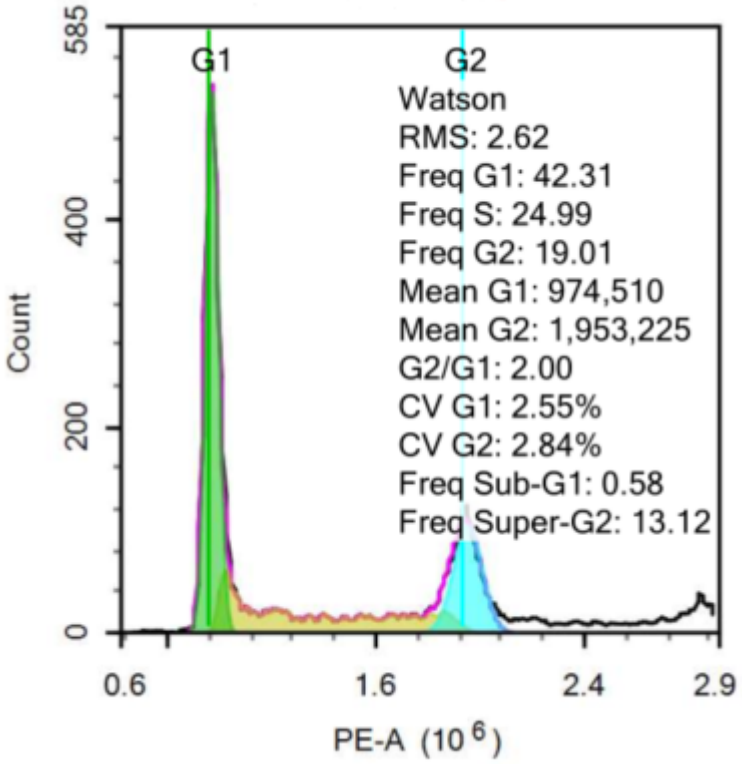
8. Add 400 µl of Staining Working Solution to stain. Incubate cells at 37 °C for 30 minutes, protected from light. If not used immediately, store the samples at 4°C, protected from light.

9. Add 2 ml PBS and centrifugate cells at 300~400 x for 5 minutes. Remove the supernatant carefully and resuspend cells with 500 µl PBS.

10. Observe at 488 nm of excitation wavelength and acquire light scatter parameters by flow cytometry within 24 hours.

11. During flow cytometry data analysis, select the main cell population in the FSC vs SSC plot. Within the main cell population, gate on single cells in the FL2-A vs FL2-W plot to exclude the cell debris and cell aggregate. Perform FL2-A histogram of single cells. Cell cycle status can be quantified by programs within the flow cytometer software or gating on the FL2-A histogram.

V. TYPICAL DATA



Cell cycle analysis of HeLa cells on NovoCyte Flow Cytometer (Agilent)