

Instructions for Use

Version: 1.0.1
Revision date: 12-Aug-25

Cell Cycle Assay Kit

Catalog No.: abx298909

Size: 100 tests

Storage: Store RNase A Reagent at -20°C. Store the PI Reagent at 2-8°C in the dark.

Application: For quantification of cell cycle progression in cell samples.

Introduction

Abbexa's Cell Cycle Assay Kit is an assay kit designed for the determination of cell cycle progression using a flow cytometer. Propidium Iodide (PI) binds DNA, and the relative DNA content of cells can be determined by flow cytometry. By measuring the fluorescence using flow cytometry, the number of G0/G1 phase cells, G2 phase cells, S phase cells and apoptotic cells can be distinguished.

Kit components

1. PI Reagent: 4 ×10 ml
2. RNase A Reagent: 10 ml

Materials required but not provided

1. Flow cytometer (Ex 535 nm, Em 617 nm)
2. Centrifuge
3. Absolute ethanol (-20°C)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vials/tubes
7. Water bath
8. Incubator

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Protocol

A. Preparation of Reagents

- **RNase A Reagent:** Allow to thaw completely before use, mix thoroughly and keep on ice.
- **Absolute ethanol:** Cool to -20°C overnight.

Sample Preparation:

- **Cell samples:** Collect at least 5×10^5 cells and centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant. Add 1 ml of PBS (0.01 M, pH 7.4) to resuspend the cells gently. Centrifuge at $300 \times g$ for 5 minutes and discard the supernatant. Add 0.3 ml PBS (0.01 M, pH 7.4) to resuspend the cells. Add into 1.2 ml of absolute ethanol (pre-cooled to -20°C) and store at -20°C for 1 hour or overnight.

B. Assay Procedure

1. Centrifuge the cooled cell samples at $300 \times g$ for 5 minutes and discard the supernatant. Add 1 ml of PBS (0.01 M, pH 7.4) to resuspend the cells and leave to stand at room temperature for 15 minutes.
2. Centrifuge at $300 \times g$ for 5 minutes and discard the supernatant. Add 100 µl of RNase A Reagent to resuspend the cells. Incubate in a 37°C water bath for 30 minutes.
3. Add 400 µl of PI Reagent, mix thoroughly, then incubate at 2-8°C for 30 minutes in the dark.
4. Measure the fluorescence of the cell suspension using a flow cytometer, with an excitation maximum of 535 nm and an emission maximum of 617 nm.

C. Results

Following staining with PI, fluorescence intensity measured by flow cytometry correlates with DNA content in different phases of the cell cycle. Use the following theoretical values to identify cell populations:

Cell Cycle Phase	DNA Content	Relative Fluorescence Intensity (FI)	Interpretation
G0/G1 Phase	2N (diploid)	1.0	Normal diploid DNA content (baseline)
S Phase	Between 2N and 4N	1.0 – 2.0	Actively replicating DNA; FI increases as replication progresses
G2/M Phase	4N (tetraploid)	2.0	Completed DNA replication; ready to divide
Sub-G1 (Apoptotic)	<2N due to DNA fragmentation	<1.0	Reduced DNA content due to apoptosis; appears as a sub-G1 peak

Notes:

- Apoptotic cells exhibit significantly reduced fluorescence due to DNA loss. Sub-G1 peaks on flow cytometry histograms indicate apoptotic cell populations.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.