# Cell Cycle Assay Kit

**Catalog No.:** abx295117

**Size:** 100 tests

**Storage:** Store all components at -20°C. Store the Chromogenic Reagent 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. A blue fluorescent probe 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. Chromogenic Reagent: 4 ×10 ml
2. RNase A Reagent: 10 ml

### Materials required but not provided

1. 96-well microplate, PCR plate, or equivalent
2. Flow cytometer (Ex. 364 nm, Em. 454 nm)
3. Centrifuge
4. Absolute ethanol (cooled to -20°C)
5. PBS (0.01 M, pH 7.4)
6. Pipette and pipette tips
7. Vials/tubes
8. Water bath
9. Incubator

## 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 × 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 × g for 5 minutes and discard the supernatant. Add 0.3 ml of PBS (0.01 M, pH 7.4) to resuspend the cells. Add into 0.7 ml of absolute ethanol (pre-chilled to -20°C) and store at -20°C for 1 hour or overnight.

### B. Assay Procedure

1. Centrifuge the prepared cell sample at 300 × 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 × 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 Chromogenic Reagent, mix thoroughly, then incubate at 2-8°C for 30 minutes in the dark.
4. Measure the fluorescence of the cells using a flow cytometer, with an excitation maximum of 364 nm and an emission maximum of 454 nm.

### C. Results

Following staining with blue probe, 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.