



TECHNICAL MANUAL

Cell Cycle Assay Kit (Red Fluorescence)

- **SKU CODE:** AKES078
- **SIZE:** 20 Assays / 50 Assays / 100 Assays
- **RUO:** Research-Use-Only

Cell Cycle Assay Kit (Red Fluorescence)

Please read entire manual carefully before starting experiment.

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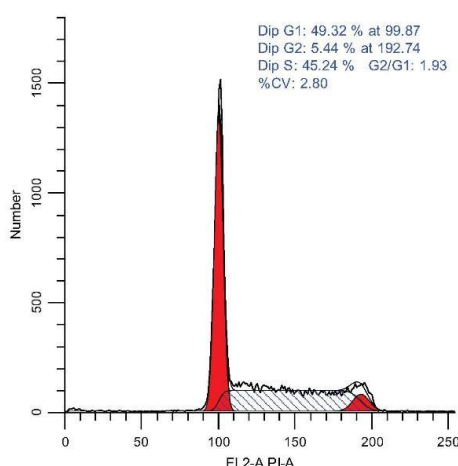
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1. Product Description

The Assay Genie Cell Cycle Assay Kit (Red Fluorescence) is designed for the analysis of the cell cycle by measuring cellular DNA content using flow cytometry.

The cell cycle refers to the whole process from the end of one mitosis to the end of the next. During this process, the genetic material is replicated and doubled, and evenly distributed to two daughter cells at the end of division. The cell cycle can be divided into phases such as interphase and metaphase. The intercellular phase can be further divided into dormancy (zero gap, G0), prophase of DNA synthesis (first gap, G1), DNA synthesis (S) and anaphase of DNA synthesis (second gap, G2). DNA binds to certain specific fluorescent dyes (such as propidium iodide, PI), the amount of fluorescent dye bound to DNA differs between stages, and the fluorescence intensity detected by flow cytometry can therefore be used to distinguish the different phases of the cell cycle.

After staining with PI, assuming that the fluorescence intensity of G0/G1 phase cells is 1, the theoretical fluorescence intensity of G2/M phase cells, which contain double the genomic DNA, is 2, and the fluorescence intensity of S phase cells undergoing DNA replication is between 1 and 2. Apoptotic cells lose part of their genomic DNA as a result of nuclear condensation and DNA fragmentation. Therefore, apoptotic cells show obvious weak staining after PI staining and their fluorescence intensity is less than 1. The sub-G1 peak that appears in the flow cytometry result corresponds to apoptotic cells.



Molt-4 cells were treated with ethanol overnight and detected with this kit.

2. Kit Contents

Component Name	20 Assays	50 Assays	100 Assays	Storage
RNase A Reagent	10 mL × 1	10 mL × 1	10 mL × 1	-20°C
PI Reagent (50 µg/mL)	10 mL × 1	10 mL × 2	10 mL × 4	2~8°C, protect from light
Manual	One Copy			

3. Storage & Expiry

- RNase A Reagent should be stored at -20°C.
- PI Reagent (50 µg/mL) should be stored at 2~8°C, protected from light.
- For maximal assay performance, this kit should be used within 12 months. Avoid freeze/thaw cycles.

4. Experimental Procedure

Materials required but not supplied: absolute ethanol, PBS, a refrigerated centrifuge, a 37°C water bath and a flow cytometer.

1. Reagent Preparation

- 1) Store the absolute ethanol at -20°C overnight.
- 2) Take out the RNase A Reagent, allow it to dissolve fully, mix it and place it on ice for use.

2. Sample Preparation

- 1) Collect 5×10^5 cells for each sample, centrifuge at $300 \times g$ for 5 min and discard the supernatant. Add PBS to resuspend the cells gently and count them.
- 2) Centrifuge at $300 \times g$ for 5 min and discard the supernatant.
- 3) Add 0.3 mL PBS to resuspend the cells.

3. Add 1.2 mL absolute ethanol from the -20°C freezer, mix fully and store at -20°C for 1 h or overnight.
4. Centrifuge at 300×g for 5 min and discard the supernatant. Add 1 mL PBS to resuspend the cells and store at room temperature for 15 min.
5. Centrifuge at 300×g for 5 min and discard the supernatant. Add 100 µL RNase A Reagent to resuspend the cells and incubate in a 37°C water bath for 30 min.
6. Add 400 µL PI Reagent (50 µg/mL), mix fully and incubate at 2-8°C for 30 min in the dark.
7. Analyse the cells immediately using appropriate flow cytometer settings.

5. Important Notes

1. For maximal assay performance, this kit should be used within 12 months. Avoid freeze/thaw cycles.
2. The experimental results need to be detected by flow cytometry.
3. Detect apoptosis as soon as possible after staining to avoid an increase in apoptosis or necrosis.
4. Avoid extended exposure of the samples to direct light in order to protect the fluorophores from quenching.
5. This kit is for research use only. For your safety and health, please wear a laboratory coat and gloves. The instructions should be followed strictly; changes to the procedure may result in unreliable results.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

