# Product Information

## Cell Cycle Assay Kit Plus

Catalog Number: C6078S, C6078

Product Size: 20T, 50T

Contents:

| Component | 20T | 50T |
|---|---|---|
| A. Binding buffer (10×) | 4 mL | 10 mL |
| B. RedNucleus I Staining Solution | 80 μL | 200 μL |

## Storage

Store at 4°C. Expiration date marked on the outer packing. For long-term storage, please store at -20°C. RedNucleus I should be protected from light.

## Description

The upgraded version of the cell cycle detection kit can be used for live and fixed cell cycle assays with certain applicability. The cell types currently validated by our company are Hela, Molt-4, Jurkat, and K562. For Hela and Molt-4, both live cells and 75% ice ethanol overnight fixation at 20°C could be detected, but for the other two types of Jurkat and K562, both live and fixed states could not be detected. Therefore, the suitability of the assay for different cell types needs to be determined after testing.

The upgraded version of the cell cycle detection kit uses RedNucleus I staining method to detect the cell cycle. RedNucleus I is a far-infrared nucleic acid dye with cell membrane permeability, which can quickly enter living cells, specifically bind DNA and detect the cycle of live cells without RNase digestion. Compared with the traditional propidium iodide staining method, the cells do not need membrane rupture or fixation, and the operation is simpler.

RedNucleus I is a fluorescent dye of double stranded DNA. The fluorescence intensity after binding with double stranded DNA, and is directly proportional to the content of double stranded DNA. The intracellular DNA content can be measured by flow cytometry, and then the cell cycle can be analyzed according to the distribution of DNA content. After RedNucleus I staining, assuming that the fluorescence intensity of $G_0$ / $G_1$ phase cells is 1, the theoretical value of fluorescence intensity of $G_2$ / M phase cells containing double genomic DNA is 2, and the fluorescence intensity of S phase cells undergoing DNA replication is between 1-2. In addition, RedNucleus I is compatible with dyes such as Horizon BV/BUV, FITC and R-PE, and can be tested periodically after sample dyeing.

This kit is usually used for cell cycle detection of cultured adherent or suspended cells. If it is used for cell cycle detection of tissue, the tissue must be digested into a single-cell state first.





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## Protocol

**Experimental materials**

- Cell lines or other cell samples (self prepared)
- Cell Cycle Assay Kit Plus
- Trypsin (self prepared)
- Cell culture medium containing FBS (self prepared)

**Experimental procedure**

1. Preparation of cell samples

(1) (This step is for adherent cells. If it is a suspension cell, proceed directly to step (2)) Digest the cells with trypsin, add cell culture medium, gently blow off the cells, and collect them in a centrifuge tube.

Note: 50,000 cells and above are required for the upload. Therefore, the initial number of cells collected needs to be sufficient.

(2) Centrifuge at about 1000 g for 3-5 min to pellet the cells. Carefully aspirate the supernatant, add about 1 mL ice-cooled 1× staining buffer (dilute 10× staining buffer with di$H_2O$ 1:10), and resuspend the cells. Repeat.

(3) Centrifuge at about 1000 g for 3-5 min to pellet the cells. After discarding the supernatant, add 1 mL of medium to resuspend the cells (for fixed cells, 1×PBS can also be used to resuspend). Gently flick the bottom of the centrifuge tube to properly disperse the cells to avoid clumping of the cells.

2. Staining: Add 4 μL of RedNucleus I staining solution to each tube of cell samples, mix slowly and thoroughly, and incubate in the dark at room temperature for 20 min (or incubate at 37°C for 5-10 min in the dark). The optimal incubation time varies from cell to cell, and the staining time can be subsequently adjusted and optimized appropriately according to the actual staining results in order to obtain more ideal staining results.

3. Flow cytometry and analysis: Use a flow cytometer to excite at 638 nm. It is recommended to detect on the RL3 or FL4 channel, or use the RL1 and RL2 channels. Use appropriate analysis software for cell DNA content analysis and light scattering analysis.

## Notes

1. Before use, please centrifuge the product to the bottom of the tube briefly, and then perform subsequent experiments.

2. This product is suitable for live cell and fixed cell cycle detection, which has certain limitations. For different types of cells, whether it is suitable or not needs to be determined after testing. For fixation, it is recommended to use ice bath to pre-cool 75-80% ethanol at -20°C overnight to fix cells.

3. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

4. For your safety and health, please wear lab coats and disposable gloves.





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