Instructions for use:

1. Preparation of cell samples:

a. For adherent cells: carefully collect the cell culture medium into a centrifuge tube for later use. The cells were digested with trypsin, and when the cells were gently pipetted with a pipette or a pipette tip, the previously collected cell culture medium was added, all adherent cells were blown, and the cells were gently blown off. Collect again into the centrifuge tube. Centrifuge the cells for about 3-5 minutes by centrifugation at 1000 g. For a specific cell, if the cell precipitation is insufficient, the centrifugation time may be appropriately extended or the centrifugal force may be slightly increased. Carefully aspirate the supernatant to leave approximately 50 microliters of culture medium to avoid aspiration of cells. Add about 1 ml of ice bath to pre-cooled PBS, resuspend the cells, and transfer to a 1.5 ml centrifuge tube. The cells were again pelleted by centrifugation, and the supernatant was carefully aspirated, leaving about 50 μl of PBS remaining to avoid aspiration of the cells. Gently bomb the bottom of the tube to properly disperse the cells to avoid cell agglomeration.

b. For suspended cells: centrifuge for about 3-5 minutes for about 3-5 minutes to pellet the cells. For a specific cell, if the cell sedimentation is insufficient, the centrifugation time may be appropriately extended or the

centrifugal force may be slightly increased. Carefully aspirate the supernatant to leave approximately 50 microliters of culture medium to avoid aspiration of cells. Add about 1 ml of ice bath to pre-cooled PBS, resuspend the cells, and transfer to a 1.5 ml centrifuge tube. The cells were again pelleted by centrifugation, and the supernatant was carefully aspirated, leaving about 50 μl of PBS remaining to avoid aspiration of the cells. Gently bomb the bottom of the tube to properly disperse the cells to avoid cell agglomeration.

2. Cell fixation: Add 1 ml of ice bath to pre-cool 70% ethanol, mix gently by pipetting, and fix at 4 °C for 2 hours or longer. Fixing for 12-24 hours may work better.

Centrifuge the cells for about 3-5 minutes by centrifugation at 1000 g. For a specific cell, if the cell precipitation is insufficient, the centrifugation time may be appropriately extended or the centrifugal force may be slightly increased. Carefully aspirate the supernatant to leave approximately 50 microliters of 70% ethanol to avoid aspiration. Add about 1 ml of ice bath pre-chilled PBS and resuspend the cells. The cells were again pelleted by centrifugation, and the supernatant was carefully aspirated, leaving about 50 μl of PBS remaining to avoid aspiration of the cells. Gently bomb the bottom of the tube to properly disperse the cells to avoid cell agglomeration.

3. Preparation of propidium iodide staining solution: Refer to the table below to prepare an appropriate amount of propidium iodide staining solution according to the number of samples to be tested:

	1 sample	6 samples	12 samples
Staining buffer	0.5ml	3ml	6ml
Propidium iodide staining solution (20X)	25μl	150μl	300μl
RNase A(50X)	10μl	60μl	120μl
Final volume	0.535ml	3.21ml	6.42ml

Note: The prepared propidium iodide staining solution can be stored at 4 ° C for a short time, and should be used on the same day.

4. Staining: 0.5 ml of propidium iodide staining solution was added to each tube cell sample, and the cell pellet was slowly and fully resuspended, and kept at 37 ° C for 30 minutes in the dark. It can then be

stored in the dark at 4 ° C or in an ice bath. After the dyeing is completed, the flow detection should be completed within 24 hours, and it is better to complete the flow detection on the same day.
5. Flow detection and analysis: The red fluorescence was detected by a flow cytometer at an excitation wavelength of 488 nm, and the light scattering was detected. Cellular
DNA content analysis and light scattering analysis were performed using appropriate analysis software .