Cell Cycle Staining Kit

Cat #: KTA2020 Size: 50 T/100 T

	Cell Cycle Staining Kit		
REF	**Cat #:** KTA2020	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

The most common approach to determining the cell cycle stage (G1, S, and/or G2/M) is based on measurement of cellular DNA content. As cells progress through the cell cycle, cells in the G1 phase have one set of paired chromosomes and are uniform with respect to DNA content. On the other hand, the amount of DNA begins to double during S phase, so that the amount of DNA is between one and two times the amount in G1. Cells in G2/M phase have double the amount of DNA compared to cells in G1 and two sets of paired chromosomes.

This kit utilizes a nuclear dye, the binding of which to nucleic acids in the cell results in fluorescence signal, which is proportional to cellular DNA content, providing a convenient and accurate determination of the percentage of cells in each phase of the cell cycle.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	100 T	
Nuclear Dye (50×)	0.5 mL	1mL	-20℃, protected from light
Assay Buffer (10×)	2.5 mL	5 mL	4℃
RNase A (100×)	0.25 mL	0.5 mL	-20℃

Materials Required but Not Supplied

- Microcentrifuge
- Precision pipettes, disposable pipette tips
- Flow cytometer
- Deionized water, PBS, 70% ethanol

Reagent Preparation

Nuclear Dye (50×): Warm to room temperature. Aliquot and store unused solutions at -20℃. Protect from light and avoid repeated freeze-thaw cycles.

1×Assay Buffer: Prepare 1×Assay Buffer by dilute 10×Assay Buffer with Deionized Water. Pre-chill 1×Assay Buffer on ice before use.

Staining Solution: Before performing the analysis, prepare Staining Solution, add 100 µL of RNase A Solution and 200 µL of Nuclear Dye into 10 mL of 1×Assay Buffer, mix well and protect from light. Stable for one week at 4℃.

Assay Procedure

Note: The optimal concentration of the Nuclear Dye and incubation time varies depending on the specific application. The staining conditions may need modified according to the particular cell type.

1. Treat cells with the desired method.
2. For non-adherent cells, collect 1-2×10^6 cells by centrifugation (4℃, 600 g, 5 min). For adherent cells, using Trypsin to digest cells firstly and then centrifugation.
3. Wash cell pellet with ice-cold PBS twice.
4. Centrifuge the cells 600 g for 5 min and transfer the tubes to ice.
5. Slowly resuspend the cells with ice-cold 70% ethanol in deionized water. Place cells at -20℃ for 2 h or longer (It is recommended overnight fixation, 12-24 h). These cells can be kept for weeks at -20℃ before staining and analysis.

Note: It is very important that the cells are fixed into a single cell suspension. Cells can tend to clump during fixation. Very slow, drop-wise addition of the initial volume of 70% ethanol while gently vortexing helps prevent cells from clumping.

6. Centrifuge the cells at 1,000 g for 5 min at 4℃.
7. Remove the ethanol and resuspend cells in 1 mL of ice-cold PBS.
8. Centrifuge cells at 500 g for 10 min at 4℃ and remove PBS. Repeat step 7 and 8 twice to remove the ethanol thoroughly.
9. Resuspend the cells in 0.5 mL Staining Solution and incubate the cells at 37℃ for 30 min, in the dark.

Wash cells with PBS twice and resuspend with ice-cold PBS. Analyze the cells within 24 h by flow cytometry using the appropriate channel (Ex/Em=535/615 nm).

Recommended Products

Catalog No.	Product Name
KTA1001	Live and Dead Cell Double Staining Kit
KTA1002	Live Cell Tracking Kit (Green Fluorescence)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.