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Live and Dead Cell Double Staining Kit

Cat #: KTA1001 Size: 100 T/500 T/2000 T

	Live and Dead Cell Double Staining Kit		
REF	**Cat #:** KTA1001	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Cell-mediated cytotoxicity is an important phenomenon characterized by cytolysis of a compromised cell in the body by immune system. Distinguishing between live and dead cells is very important for investigation of growth control and cell death. The Live/Dead Cell Double Staining Kit provides a convenient assay to evaluate the viability of cells, based on the simultaneous determination of live and dead cells with two probes that measure recognized parameters of cell health: plasma membrane integrity and intracellular esterase activity. The kit utilizes Ca-AM, a cell-permeable green fluorescent dye (Ex/Em=488/530 nm), to stain live cells and PI, a cell non-permeable red fluorescent dye (Ex/Em=535/617 nm), to stain dead cells.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	100 T	500 T	2000 T	
Ca-AM	50 μL	250 μL	1 mL	-20℃, protected from light
PI	50 μL	250 μL	1 mL	-20℃, protected from light
Assay Buffer (10×)	5 mL	25 mL	100 mL	4℃

Materials Required but Not Supplied

- Microcentrifuge
- 24 well plate, Precision Pipettes, Disposable Pipette Tips
- Fluorescence Microscopy or Flow Cytometer
- Deionized Water, PBS

Reagent Preparation

Ca-AM: Keep on ice while using. Protect from light.

PI: Keep on ice while using. Protect from light.

1×Assay Buffer: Prepare 1×Assay Buffer by dilute 10× Assay Buffer with Deionized Water. Warm to 37℃ before use.

Staining Solution: Mix 1 μL Ca-AM and 1 μL PI in each 1 mL Assay Buffer. Scale up accordingly for larger numbers of assays.

Assay Procedure

A. Quantification by Flow Cytometry

1. Treat cells with the desired method.

Note: We recommend keeping unstained control cells (i.e. without Ca-AM or PI staining) suspended in 1×Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

2. For non-adherent cells, Collect 1-5×10^5 cells by centrifugation (4℃, 300 g, 5 min). Wash with ice-cold PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation.
3. Resuspend the cells pellet in 500 uL Staining Solution.
4. Incubate the cells at 37℃ for 15-30 min in the dark.
5. Analyze the cells by flow cytometry.

B. Detection by Fluorescence Microscopy

1. For suspension cells: Follow the protocol for flow cytometry from step A.1 to step A.4 and place the cell suspension from Step A.4 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible.
2. For adherent cells: the suggested protocol is as below.

(1) Grow cells directly on a coverslip in 24 well plate. Incubate in a CO_2 Incubator at 37℃ for at least 24 h before treatment.
(2) Treat cells with the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
(3) Wash cells with PBS twice.
(4) Add 0.5 mL of Staining solution to cells and incubate at 37℃ for 15-30 min in the dark.
(5) Wash cells with PBS twice.
(6) Invert coverslip on a glass slide and visualize cells fluorescence microscopy using the appropriate filters as soon as possible.

Note: PI is a potential mutagen. Use appropriate precautions when handling this reagent.

Recommended Products

Catalog No.	Product Name
KTA2020	Cell Cycle 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.