Live and Dead Cell Double Staining Kit

Cat #: KTA1001 Size: 100 T/500 T/2500 T

	Live and Dead Cell Double Staining Kit		
REF	**Cat #:** KTA1001	LOT	**Lot #:** Refer to product label
	Applications: Flow Cytometry and Fluorescence Microscopy detection for cell samples		
	Fluorescence Excitation/Emission: Ca-AM: Ex/Em=494/517 nm, PI: Ex/Em=535/617 nm		
	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=494/517 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	2500 T	
Ca-AM	10 μL	50 μL	250 μL	-20℃, protected from light
PI	10 μL	50 μL	250 μL	-20℃, protected from light
Assay Buffer	10 mL	50 mL	100mL×2+50mL	4℃

Materials Required but Not Supplied

- Microcentrifuge
- Cell culture plate,Precision Pipettes, Disposable Pipette Tips
- Fluorescence Microscopy or Flow Cytometer
- PBS,DMSO

Reagent Preparation

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

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

Assay Buffer: Warm to 37℃ before use.

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Staining Solution:

Flow cytometry analysis: After thawing at room temperature, we recommend using the "two-step dilution method" to prepare the staining working solution, with final dilution ratios of 1:80,000 for Ca-AM and 1:4,000 for PI.

Fluorescence microscopy analysis: Add 1 µL of Ca-AM and 1 µL of PI to each 1 mL of assay buffer.

Note: The staining solution should be prepared immediately before use. Once prepared, store it on ice away from light and use it as soon as possible. Once the dye has been diluted with assay buffer to form a working solution, it should not be left standing for too long.

Assay Procedure

A. Analysis by Flow Cytometry

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

1. Induce cell death using the desired method while culturing an uninduced control group.

2. For non-adherent cells, Collect 1-5×10^5 cells by centrifugation (4℃,1000 rpm, 5 min). Wash with PBS once and discard the PBS.

Note: After digesting adherent cells with trypsin (EDTA-free), centrifuge the cells to collect them. If the digestion time is too long, it can damage the cell membrane structure and lead to false-positive results due to cell necrosis; therefore, the trypsin digestion time must be carefully controlled (the use of a mild digestion solution is recommended).

3. Preparation of the Staining Solution:

After thawing at room temperature, it is recommended to prepare the staining working solution using the "two-step dilution method," with final dilution ratios of 1:80,000 for Ca-AM and 1:4,000 for PI. The specific procedure is as follows: First, pre-dilute the stock solutions using DMSO, diluting the Ca-AM stock solution 800-fold and the PI stock solution 40-fold. Next, add 10 µL of the pre-diluted Ca-AM and 10 µL of the pre-diluted PI to each 1 mL of Assay Buffer, mix thoroughly, and the staining working solution is ready.

Note: After pre-diluting the dye with DMSO in the first step, the remaining reagent can be stored long-term at -20°C away from light. Alternatively, the dye can be pre-diluted with Assay Buffer, but once diluted, it must be used immediately and cannot be stored long-term. The final Staining Solution must be prepared immediately before use. The amount of dye used can be adjusted based on the number and type of cells.

4. Resuspend the cells pellet in 500 uL Staining Solution.

5. Incubate the cells at 37℃ for 15-30 min in the dark.

6. After incubation, allow the sample to reach room temperature. Wash the cells once with PBS (optional). Analyze the cells by flow cytometry within 30 minutes of staining. Use 494 nm and 535 nm for excitation, and measure fluorescence at approximately 517 nm (FITC channel) and 617 nm (PE channel).

B. Detection by Fluorescence Microscopy

1.Treat cells with the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2.Cell Processing

(One)For suspension cells

(1) Collect cells by centrifugation (4℃, 1000 rpm, 5 min). Wash with PBS twice and discard the PBS.

(2) Preparation of Staining Solution: After thawing at room temperature, add 1µL of Ca-AM and 1µL of PI to each 1 mL of Assay Buffer.

Note: The amount of dye used can be adjusted based on the number and type of cells.

(3) Resuspend the cells in 500µL Staining solution and incubate at 37℃ for 15-30 min in the dark.

(4) Place the stained cell suspension on a microscope slide, cover the cells with a coverslip, and analyze the cells as soon as possible using a fluorescence microscope with the appropriate filter. (For Ca-AM, use the FITC setting; for PI, use the Cy®3 or Texas Red® setting.)

(Two)For adherent cells

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(1) Wash cells with PBS once and discard the PBS.

(2) Preparation of Staining Solution: After thawing at room temperature, add 1μL of Ca-AM and 1μL of PI to each 1 mL of Assay Buffer.

(3) Add an appropriate amount of staining solution to the cells and incubate in the dark at 37°C for 15–30 minutes.

Reagents	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Staining solution (μL)	100	150	200	300	500

(4) Place the stained cell suspension on a microscope slide, cover the cells with a coverslip, and analyze the cells as soon as possible using a fluorescence microscope with the appropriate filter. (For Ca-AM, use the FITC setting; for PI, use the Cy®3 or Texas Red® setting.)

Note: Optimize imaging parameters using a single field before formal acquisition; once set, capture all fields promptly—CA-AM fluorescence is highly photolabile and must be imaged immediately for each field.

Typical Data

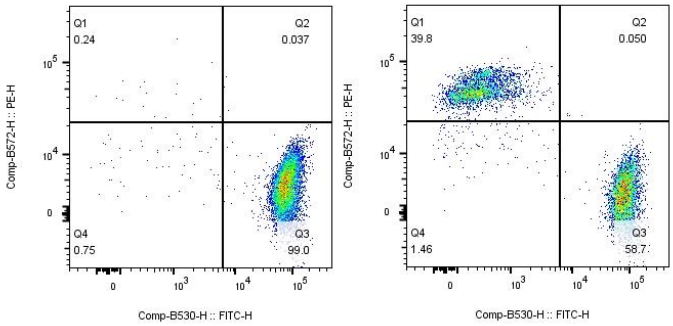


Figure 1. Untreated Jurkat cells (left) and drug-treated dead cells (right), stained using a live/dead cell dual-staining kit. The stained samples were analyzed by flow cytometry, with Ca-AM detected via the FITC channel and PI via the PE channel. The cell clusters were primarily divided into live cells (Q3) and dead cells (Q1).

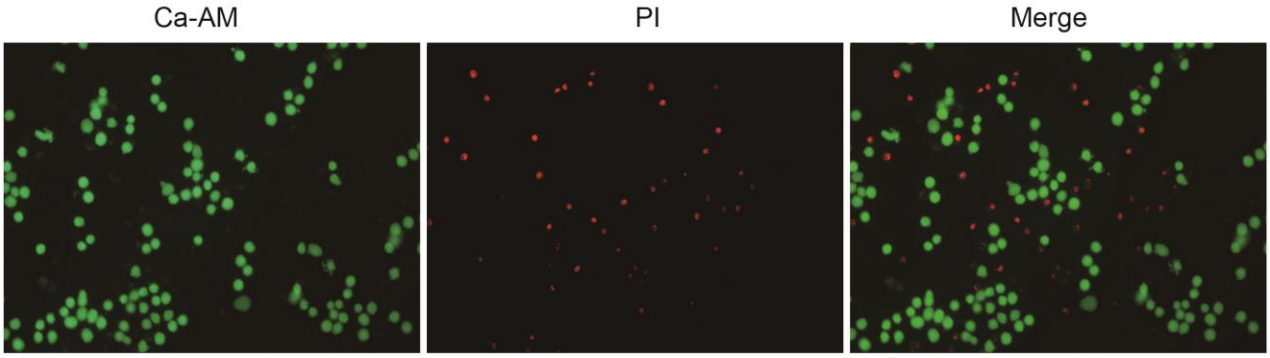


Figure 2. Mouse BMSCs were treated with Raptinal for 20 minutes and then stained using a live/dead cell dual-staining kit. This image shows the results of examining the stained samples under a fluorescence microscope.

Recommended Products

Catalog No.	Product Name
KTA2020	Cell Cycle and Apoptosis Detection Kit (Red Fluorescence)
KTA1002	Live Cell Tracking Kit (Green Fluorescence)

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KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA2011	One-step TUNEL Apoptosis Assay Kit (Red Fluorescence)
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit
KTA0006	Annexin V-FITC/PI Apoptosis Detection kit
KTA0007	Annexin V-APC/7-AAD Apoptosis Detection Kit
KTA4001	Annexin V-AbFluor™ 405 Apoptosis Detection kit (Blue Fluorescence)

Disclaimer

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

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