Elabscience® One-step TUNEL Flow Cytometry Apoptosis Kit

Catalog No: E-CK-A420/E-CK-A421/E-CK-A422/E-CK-A423/E-CK-A424/E-CK-A425

Product size: 20 Assays/50 Assays/100 Assays

Components

Cat.	Products	20 Assays	50 Assays	100 Assays	Storage
E-CK-A32A	TdT Equilibration Buffer	9 mL	20 mL	40 mL	-20°C
E-CK-A32B	TdT Enzyme	100 μL×2	250 μL×2	250 μL×4	-20°C
E-CK-A420C/ E-CK-A421C/ E-CK-A422C/ E-CK-A423C/ E-CK-A424C/ E-CK-A425C*	Labeling Solution(FITC)/ Labeling Solution(Elab Fluor® 488)/ Labeling Solution(Elab Fluor® 594)/ Labeling Solution(Elab Fluor® Violet 450)/ Labeling Solution(Elab Fluor® 647)/ Labeling Solution(Elab Fluor® 555)	200 μL×2	200 μL×5	200 μL×10	-20°C, shading light
E-CK-A42D	Fixation Buffer	6 mL	12.5 mL	25 mL	-20°C
E-CK-A42E	Permeabilization Buffer	6 mL	12.5 mL	25 mL	-20°C
E-CK-A42F	Stop Solution	20 mL	50 mL	100 mL	-20°C
Manual	One copy				

*Labeling Solution: Each catalog corresponds to a different fluorescein.

Introduction

Elabscience® One-step TUNEL Flow Cytometry Apoptosis Kit applies a highly sensitive, fast and simple method to detect cell apoptosis. This kit can be used to detect apoptosis of suspension cells and adherent cells by flow cytometry.

Detection Principle

When cells undergo apoptosis, specific DNA endonucleases will be activated, cutting the genomic DNA between the nucleosomes. The DNA of apoptotic cells is cleaved into multimers of 180~200bp fragments, corresponding to the oligonucleosomal size. Therefore, the DNA of apoptotic cells typically migrates as a ladder of 180~200bp on an agarose gel. After fixation and permeabilization, the exposed 3'-OH of the broken DNA can be catalyzed by Terminal Deoxynucleotidyl Transferase (TdT) with fluorescein labeled dUTP, and detected by flow cytometry.

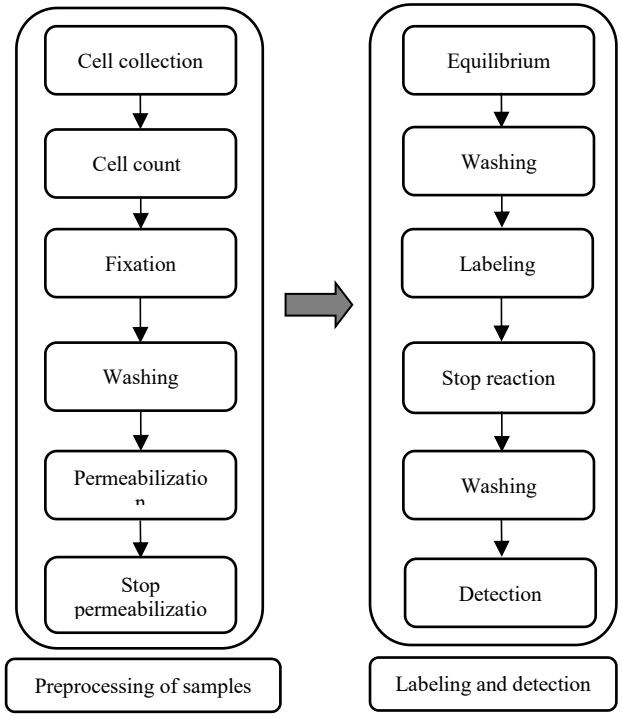
Detection Sample Types

☑ **Suspension Cells** ☑ **Adherent Cells**

Storage

The kit can be stored at -20°C for 12 months. Labeling Solution should be stored in the dark. It is recommended to aliquot the Fixation Buffer and Permeabilization Buffer into smaller quantities and avoid freezing and thawing cycles.

Assay Procedure



Materials Not Supplied

1) **Reagent**
   PBS buffer (with 1%BSA) (pH7.2 ~ 7.4).
   Note: PBS (with 1% BSA) buffer (pH7.2~7.4) is used to reduce cell loss caused by centrifugation.
2) **Instrument**
   Flow cytometry, Centrifuge.

Experimental Protocol

Notice: Negative control should be set up to show the objectivity and accuracy of TUNEL. It is recommended to set up a negative control in each experiment.

1. Collect the cell and count the cells. Take 1×10^6 cells, centrifuge at 300×g for 5 min, discard the supernatant.
   Note: If the sample is adherent cell, some apoptotic cells will be suspended in the supernatant, and these cells should also be collected.
2. Add 200 μL of PBS (with 1% BSA) to resuspend the cells, add 200 μL of Fixation Buffer, and incubate at room temperature for 60 min (Mix every 15 min).
3. Add 1mL of PBS (with 1% BSA), centrifuge at 600×g for 5 min, discard the supernatant.
4. Add 200 μL Permeabilization Buffer, incubate on ice for 10 min.
   Note: If the temperature of permeabilization is too high or the time of permeabilization is too long, the cells will be broken. Therefore, it is recommended that the Permeabilization Buffer be thawed and placed at 4°C and taken out before use. In addition, the maximum permeabilization time should not exceed 15 min.
5. Add 1~2 mL of PBS (with 1% BSA) to stop reaction, mix by gently pipetting, and then centrifuge at 600×g for 5 min, discard the supernatant.
6. Add 200 μL TdT Equilibration Buffer, mix by gently pipetting, incubate at 37°C for 15 min.
7. Centrifuge at 600×g for 5 min, discard the supernatant, and collect the cell pellet.
8. Prepare the Labeling Working Solution according to the number of samples. Please refer to the table below (100 μL of Labeling Working Solution for a sample and prepare the fresh solution before use).

Component	Experimental group	Negative Control
TdT Equilibration Buffer	70 µL	80 µL
Labeling Solution	20 µL	20 µL
TdT Enzyme	10 µL	0 µL

Note:

① Bring the TdT Equilibration Buffer to RT until the liquid completely dissolved and mix fully before use. It's a normal phenomenon that TdT Equilibriation Buffer crystallize after melting.

② Before using Labeling Solution, please dissolve it on ice and use it after mix fully.

③ TdT Enzyme is sensitive to temperature, please store it strictly at -20°C. Take it out before use and put it back immediately after use.

④ Gently pipette the Labeling Working Solution to incorporate the TdT enzyme. Stirring by vortex is not recommended.

9. Incubate at 37°C for 60 min in the dark (mix cells gently every 20 min).
10. Add 1 mL of Stop Solution, mix gently by pipetting, incubate at room temperature for 5 min, add 300~500µL PBS (with 1% BSA), centrifuge at 600×g for 5 minutes, and discard the supernatant.
11. Add 200 µL of PBS (with 1% BSA) to resuspend the cells, and detect by flow cytometry.

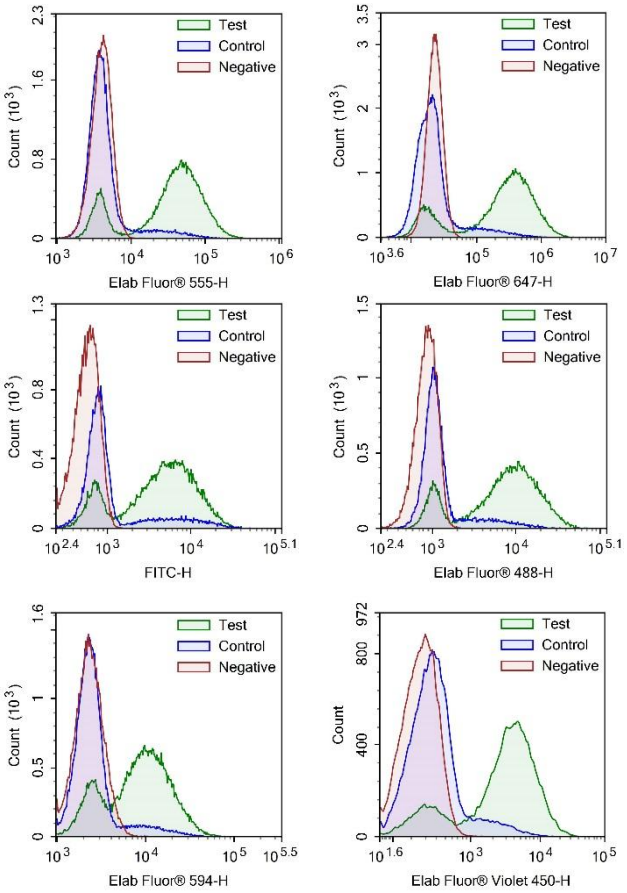
Analyze

Select the appropriate channel for detection in the flow cytometer.

Cat.No.	Dye	Ex/Em (nm)	Filter Set
E-CK-A420	FITC	490/530	FITC
E-CK-A421	Elab Fluor® 488	495/520	FITC
E-CK-A422	Elab Fluor® 594	590/617	PE-TR
E-CK-A423	Elab Fluor® Violet 450	410/450	Pacific Blue
E-CK-A424	Elab Fluor® 647	650/670	APC
E-CK-A425	Elab Fluor® 555	555/565	PE/PE-TR

Note: Please detect as soon as possible to avoid easy quenching of fluorescence.

Typical Results



Test: HL-60 cells were treated with 2.5 µM Camptothecin for 4h.

Control: Normal HL-60 cells were not treated with drugs.

Negative: HL-60 cells were treated with 2.5 µM camptothecin for 4h without TdT enzyme.

Troubleshooting

Symptoms	Causes	Comments
More cellular debris	Insufficient fixation, the cells were broken after permeabilization.	Properly prolong the fixation time and fully fix the cells before permeabilization.
	The permeabilization time is too long, or the permeabilization temperature is too high.	The permeabilization time should be controlled between 10-15 min, and incubate the samples on ice.
No apoptotic cell population detected	Failure to induce cell apoptosis.	Set different concentration gradients and induction times to find suitable induction conditions.
	The number of cells is large, so the permeabilization is insufficient.	Appropriately adjust the number of cells, or increase the dose of Permeabilization Buffer.
	Wash the cells with the buffer containing chelating agent such as EDTA, which make the TdT enzyme ineffective.	Use the buffer without chelating agent.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. The minimum number of cells for this kit should not be less than 5×10^5.
4. When resuspending the cells, gently pipette the cells 10~20 times. Avoid blowing out the liquid in the pipette completely each time to avoid cell damage and excessive bubbles.
5. Be careful to remove the supernatant after centrifugation to avoid the loss of cells.
6. Don't vortex the Labeling Solution and TdT enzyme and avoid freezing and thawing cycles.