One-step TUNEL In Situ Apoptosis Kit (Red, FineTest®594)

Catalog No.: FNCK102

Size: 20 Assays / 50 Assays / 100 Assays

Storage: Store at -20°C for 12 months. Labeling Solution and DAPI Reagent (25 μg/mL) should be stored in the dark.

Cat.	products	20 Assays	50 Assays	100 Assays	Storage
FNCK10A	TdT Equilibration Buffer	4 mL	9 mL	9 mL×2	-20°C
FNCK10B	TdT Enzyme	100 μL	250 μL	250 μL×2	-20°C
FNCK10C	Proteinase K (100×)	20 μL	50 μL	100 μL	-20°C
FNCK102D	Labeling Solution(FineTest® 594)	100 μL×2	100 μL×5	100 μL×10	-20°C
FNCK10E	DNase I (20 U/μL)	5 μL	13 μL	25 μL	-20°C
FNCK10F	DNase I Buffer (10×)	300 μL	700 μL	1500 μL	-20°C
FNCK10G	DAPI Reagent(25 μg/mL)	100 μL	250 μL	500 μL	-20°C

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

Introduction

One-step TUNEL In Situ Apoptosis Kit applies a highly sensitive, fast and simple method to detect cell apoptosis. The results can be directly observed through a fluorescence microscope.

This kit is suitable for in situ apoptosis detection of tissue samples (paraffin section and frozen section) and cells (cell slide and cell smear).

Detection Principle

When cells undergo apoptosis, specific DNA endonucleases will be activated, cutting the genomic DNA between the nucleosomes. The exposed 3'-OH of the broken DNA can be catalyzed by Terminal Deoxynucleotidyl Transferase (TdT) with fluorescein labeled dUTP, which can be detected with fluorescence microscope.

Materials Not Supplied

1) Cell Sample

Fixative buffer: Polyformaldehyde dissolved in PBS with final concentration of 4%.

Permeabilization Buffer: Triton-100 dissolved in PBS with final concentration of 0.2%.

2) Paraffin Section

Xylene, ethanol.

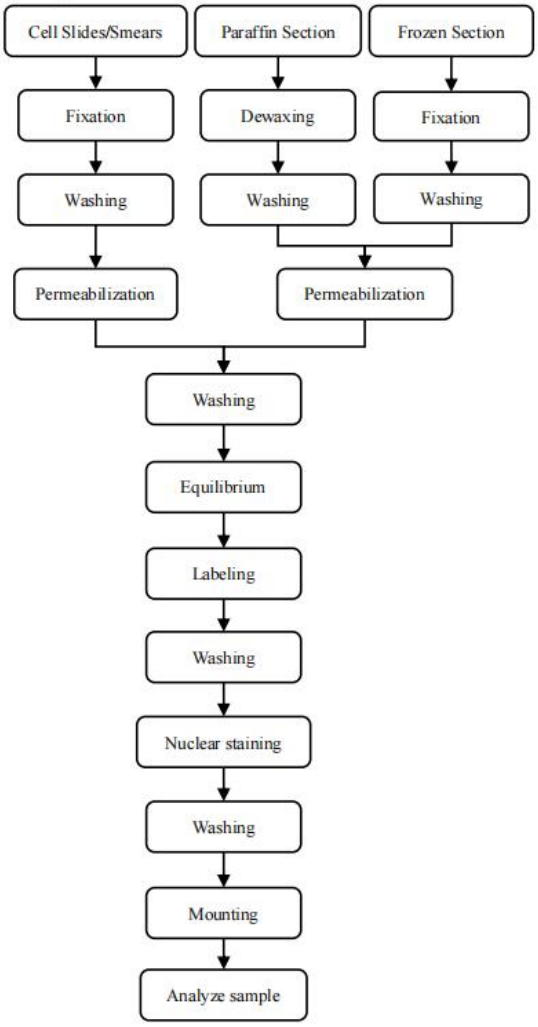
3) Frozen Section

Fixative buffer: Polyformaldehyde dissolved in PBS with final concentration of 4%.

4) Other Reagents

PBS, ddH2O, anti-fluorescence quenching agent.

Assay Procedure



Experimental procedure

Reagent Preparation

1) 1×Proteinase K working solution

Add 1 μL Proteinase K (100×) to 99 μL PBS and mix well. Prepare the fresh solution before use.

2) 1×DNase I Buffer

Dilute the DNase I Buffer (10×) with ddH_2O to 1×DNase I buffer according to the dilution ratio of 9:1. Prepare the fresh solution before use.

3) DNase I working solution (200 U/mL)

Dilute the DNase I (20 U/μL) with 1×DNase I buffer to DNase I working solution (200 U/mL) according to the dilution ratio of 99：1. Prepare the fresh solution before use.

Note: Do not vortex the DNase I as DNase I will denature with vigorous mixing.

4) DAPI working solution

Add 4 μL DAPI Reagent (25μg/mL) to 96 μL PBS and mix well. Prepare the fresh solution before use.

Fixation and Permeabilization

Sample processing

1. Cell sample

1) Cell slides: Wash the slides with PBS for 1 time. Absorb the moisture around the sample with filter paper. Immerse the cell slides into the fixative buffer (self-prepared) at RT for 15~20 min or at 4°C for 1~2 h.

Cell smears: Collect the cell, Add a certain volume of PBS to resuspend the cells and then add equal volume of fixative buffer (self-prepared) at RT for 15~20 min or at 4°C for 1~2 h, centrifuge at 600×g for 5 min. Add PBS to resuspend the cells and spread 25~50 μL cell suspension on slides and dried.

2) Wash the slides with PBS for 3 times, 5 min each time.

3) Put the slides into the Permeablilization Buffer (selfprepared), and incubate at 37°C for 10 min.

4) Wash the slides with PBS for 3 times, 5 min each time.

2. Paraffin section

1) Deparaffinize and hydrate the paraffin slides by conventional methods. Immerse slides in xylene (self-prepared) for twice, 10 min each time. Immerse slides in Anhydrous ethanol (self-prepared) for twice, 5 min each time, then hydrate the paraffin sections with a sequential of hydrated ethanol of different percentages shown as follows: 90%, 80%, 70% (self-prepared), 3 min each step.

2) Wash the slides with PBS for 3 times, 5min each time.

3) Absorb the moisture around the tissue with filter paper. Add 100 μL of 1×Proteinase K working solution to each sample, and incubate at 37°C for 20 min.

Note: The time of incubation for samples from different tissue or species may be different. It is recommended to take a preliminary experiment to confirm the incubation time.

4) Wash the slides with PBS for 3 times, 5 min each time.

3. Frozen section

1) Take out the frozen sections, equilibrium to room temperature, then immerse the frozen slides in the Fixative Buffer (self-prepared), and incubate at RT (15~25°C) for 30 min.

2) Wash the slides with PBS for 2 times, 5 min each time.

3) Add 100 μL of 1×Proteinase K working solution to each sample, and incubate at 37°C for 10~20 min.

Note: The time of incubation for samples from different tissue or species may be different. It is recommended to take a preliminary experiment to confirm the incubation time.

4) Wash the slides with PBS for 3 times, 5 min each time.

Labeling

1. Group setting

Positive control preparation

1) Add 100 μL of 1×DNase I Buffer to each slide, and incubate at RT for 5 min.

2) Carefully blot the liquid around the sample areas with absorbent paper. Add 100 μL DNase I working solution (200 U/mL) on each slide, and incubate at 37°C for 10~30 min.

3) Wash the slide with PBS for 3 times, 5 min each time.

Negative control preparation

1) Add 100 μL of 1×DNase I Buffer to each slide, and incubate at RT for 5 min.
2) Incubate the Negative sample with DNase I Buffer at 37°C for 10~30 min.
3) Wash the slide with PBS for 3 times, 5 min each time.

Experimental group preparation

1) After the experimental group completed the penetration step, it was placed in PBS and waited for the positive control and negative control to be labeled and stained together.

2. Preparation of Labeling Working Solution

Prepare the Labeling Working Solution according to the number of samples. Please refer to the table below (Prepare the fresh solution before use).

Component	Positive Control / Experimental group	Negative Control
TdT Equilibration Buffer	35 μL	40 μL
Labeling Solution	10 μL	10 μL
TdT Enzyme	5 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.
5. The sample area covered by the 50 μL Labeling working Solution is about 5 cm^2. For samples with larger surface areas, the volume of the Labeling Working Solution can be proportionally increased.

3. Labeling protocol

1) Add 100 μL of TdT Equilibration Buffer to each sample, and incubate at 37°C for 10~30 min.
2) Carefully blot the liquid around the sample areas with absorbent paper (Do not allow the samples to dry out). Add 50 μL of Labeling working solution to each slide, and incubate at 37°C for 60 min with shading light in humidified chamber.

Note: If signal intensity is low, the incubation time for the DNA-labeling reaction can be extended. Labeling times of up to 4 hours at 37°C may be required for some systems.

3) Wash the slides with PBS for 3 times, 5 min each time.
4) Carefully blot the liquid around the sample areas with absorbent paper. Add DAPI working solution, and incubate at RT for 5 min with shading light.
5) Wash the slides with PBS for 4 times, 5 min each time.
6) Carefully blot the liquid around the sample areas with absorbent paper. Add Anti-Fluorescence Quenching Agent (self-prepared) to seal the slides.

Analyze

Samples can directly be analyzed under a fluorescence microscope with appropriate filter.

Cat.No.	Dye	Ex/Em (nm)	Filter Set
FNCK100	FITC	490/520	FITC Filter Set
FNCK101	Labeling Solution(FineTest® 488)	495/519	FITC Filter Set
FNCK102	Labeling Solution(FineTest® 594)	590/617	TRITC Filter Set
FNCK103	Labeling Solution(FineTest® 647)	650/665	Cy5 Filter Set
FNCK104	Labeling Solution(FineTest® 555)	555/565	TRITC Filter Set
DAPI		350/470	DAPI Filter Set

Note: Please observe the results as soon as possible, otherwise store the slides at 4°C and protect from light.

Notes

1. After washing the slides with PBS, please blot the liquid around the sample areas with absorbent paper as much as possible before proceeding to the next step.
2. Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slides.
3. TdT enzyme working solution should be freshly prepared before use, and preserved on ice short time. Long-term storage will lead to enzyme inactivation and affect the experimental results.
4. This product is only limited to scientific research personnel, can not be used for clinical diagnosis or treatment, food or medicine, must not be stored in the ordinary residential.
5. For your safety and health, please wear a lab coat and disposable gloves during the operation.
6. The conditions recommended in this manual are universal. Users can optimize the sample processing time, reagent concentration and other conditions according to different sample types and pre-experiment results, and select the most suitable experimental conditions.