(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

One-step TUNEL In Situ Apoptosis Kit

Product size: 20Assays/50Assays/100Assays

Introduction

One-step TUNEL Assay Kit applies a highly sensitive, fast and simple method to detect cell apoptosis. This kit is suitable for in situ apoptosis detection of tissue samples (paraffin section and frozen section) and cells (cell slide and cell smear). The results can be directly observed through a fluorescence microscope.

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. 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.

Detection Sample Types

☑ **Cell Slides/Smears** ☑ **Paraffin Section** ☑ **Frozen Section**

Components

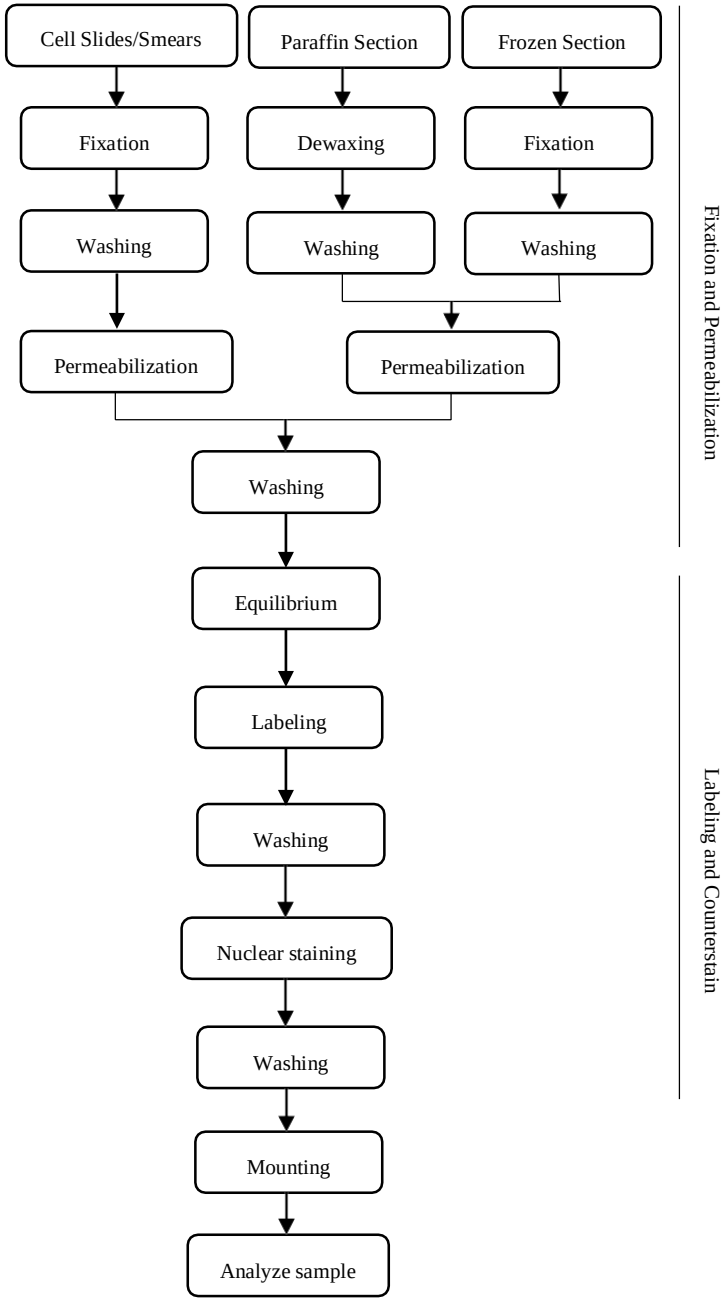
Products	20 Assays	50 Assays	100 Assays	Storage
TdT Equilibration Buffer	4 mL	9 mL	9 mL×2	-20°C
TdT Enzyme	100 µL	250 µL	250 µL×2	-20°C
Proteinase K (100×)	20 µL	50 µL	100 µL	-20°C
Labeling Solution(FITC)/ Labeling Solution(Fluor® 488)/ Labeling Solution(Fluor® 594)/ Labeling Solution(Fluor® 647)/ Labeling Solution(Fluor® 555)	100 µL×2	100 µL×5	100 µL×10	-20°C
DNase I (20 U/µL)	5 µL	13 µL	25 µL	-20°C
DNase I Buffer (10×)	300 µL	700 µL	1500 µL	-20°C
DAPI Reagent(25 µg/mL)	100 µL	250 µL	500 µL	-20°C
Manual One Copy				

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

Storage

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

Assay Procedure



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%. The prepared solution can be used after store at 4℃ for 1 ~2 days.

2) **Paraffin Section**

   Xylene, ethanol.

3) **Frozen Section**

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

4) **Other Reagents**

   PBS, ddH_2O, anti-fluorescence quenching agent.

5) **Instrument**

   Fluorescence microscope.

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. 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). 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 (25×) to 96 μL PBS and mix well. Prepare the fresh solution before use.

Fixation and Permeabilization

1. Cell sample

1) Cell slides: Wash the slides with PBS for 1 time. Absorbs 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.

   Note: Cell fixation is an important step in analyzing apoptotic samples. Unfixed cells may lose smaller DNA fragments, leading to lower signals.

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

3) Put the slides into the Permeablilization Buffer (self-prepared), 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, then hydrate the paraffin sections with a sequential of hydrated ethanol of different percentages shown as follows: 100%, 95%, 90%, 80%, 75%, 3 min each step.

   Note: Low temperature may affect the effect of xylene dewaxing. Therefore, the time of xylene dewaxing can be extended to 20 min when the room temperature is lower than 20°C .

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

3) Absorbs 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 min.

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

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

Labeling

➢ **Preparation procedure**

1. (Optional) Preparation of Positive and Negative Control

Positive and negative controls should be set up to show the objectivity and accuracy of TUNEL. It is recommended to set up a positive and a negative control in each experimental.

Note: The preparation of negative and positive control can be performed at the same time.

1) Positive control preparation

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

b) 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.

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

2) Negative control preparation

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

b) Incubate the Negative sample with DNase I Buffer at 37°C for 10~30 min.

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

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.

➢ **Labeling protocol**

1. Add 100 μL of TdT Equilibration Buffer to each sample, and incubate at 37℃ 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.

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

Troubleshooting

Symptoms	Causes	Comments
Non-specific staining	The concentration of TdT enzyme is too high.	Use TdT Equilibration Buffer to dilute 1:2~1:10.
	The time of TdT enzyme reaction is too long or the reaction solution leaks during the TdT enzyme reaction, and the cell or tissue surface cannot be kept moist.	Pay attention to control the reaction time and ensure that the TdT enzyme reaction solution can cover the sample well.
	Ultraviolet light will cause the embedding reagent to polymerize (for example, methacrylic acid will cause the fragmentation of the sample DNA).	Try to use other embedding materials or other polymerization reagents.
	The DNA of the sample is broken when the tissue is fixed (the effect of endogenous nuclease).	Ensure that the sample is fixed immediately after sampling or fixed by hepatic vein perfusion.
	Inappropriate fixatives are used, such as acidic fixatives.	Use recommended Fixative Buffer.
	Some nuclease activity is still high after fixation, causing DNA breakage.	Block with a solution containing dUTP and dAPT.
Little or poor staining	Samples fixed with ethanol or methanol (the chromatin failed to cross-link with the protein during fixation, and was lost during the operation).	Fix with 4% paraformaldehyde or formalin or glutaraldehyde dissolved in PBS pH7.4.
	Fixing time is too long, resulting in too high degree of cross-linking.	Reduce fixation time, or fix with 2% paraformaldehyde dissolved in PBS pH7.4.
	Insufficient deparaffinization of Paraffin section.	Extend dewaxing time or replace with a new dewaxing solution.
	Fluorescence quenched.	Pay attention to avoid light operation.
	The permeation promotion conditions are so poor that the reagent cannot reach the target molecule or the concentration is too low.	1. Increase the reaction time of permeabilizing agent. 2. Increase the temperature of the penetrating agent (37℃). 3. Optimize the concentration and duration of proteinase K.

High background	Mycoplasma contamination.	Use mycoplasma stain detection kit to detect whether it is mycoplasma contamination.
	The concentration of TdT enzyme is too high or the reaction time is too long.	Use TdT Equilibration Buffer to dilute 1:2~1:10 or pay attention to control the reaction time.
	The autofluorescence caused by hemoglobin in red blood cells causes serious interference.	Other apoptosis detection kits can be selected.
Positive control has no signal	The concentration of DNase I working solution is too low.	Increase the concentration of DNase I working solution.
	Insufficient washing with proteinase K.	Increase washing times or extend washing time.
	For cell samples, 0.2% Triton-100 do not mix fully.	Prepare 0.2% Triton-100 1~2 days in advance.
Loss of sample from the slides	The sample is digested by the enzyme from the slide.	Reduce the processing time of proteinase K.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. The washing operation should be sufficient, otherwise it will affect the enzyme activity (such as DNase I and TdT Enzyme) subsequent experimental operations. After washing the slides with PBS, please carefully blot the liquid around the sample areas with absorbent paper.
4. Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slides.
5. Avoid repeated freezing and thawing of the Labeling Solution and TdT enzyme. Stirring by vortex is not recommended.
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.