TUNEL In Situ Apoptosis Kit (HRP-DAB Method)

Catalog No.: FNCK105

Size: 20 Assays / 50 Assays / 100 Assays

Storage: Store at -20°C for 12 months. Streptavidin-HRP and DAB Concentrate (20×) 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
FNCK105D	Streptavidin-HRP	10 μL	25 μL	50 μL	-20°C
FNCK105E	Biotin-dUTP	100 μL	250 μL	500 μL	-20°C
FNCK105F	DAB Concentrate (20×)	200 μL	500 μL	1 mL	-20°C
FNCK105G	DAB Dilution Buffer	4 mL	10 mL	10 mL×2	-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

Introduction

TUNEL In Situ Apoptosis Kit (HRP-DAB Method) applies a highly sensitive and simple method to detect cell apoptosis.

This kit is suitable for in situ apoptosis detection of tissue samples (paraffin sections, frozen sections)and cell samples (cell smears, slides), and the detection results can be observed by optical microscope.

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 biotinlabeled dUTP, horseradish peroxidase (HRP)-labeled Streptavidin (Streptavidin-HRP) can be combined with biotin. So apoptotic cells can be observed by DAB reaction with optical microscope.

Materials Not Supplied

1. Cell Sample

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

Blocking Buffer: Dilute H_2O_2 with deionized water to a concentration of 3%.

Permeablilization Buffer: Triton-100 dissolved in PBS with final concentration of 0.2%. The prepared solution can be used after store at 4°C for 1~2 days.

2. Paraffin Section

Xylene, ethanol.

Blocking Buffer: Dilute H_2O_2 with deionized water to a concentration of 3%.

3. Frozen Section

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

Blocking Buffer: Dilute H_2O_2 with deionized water to a concentration of 3%.

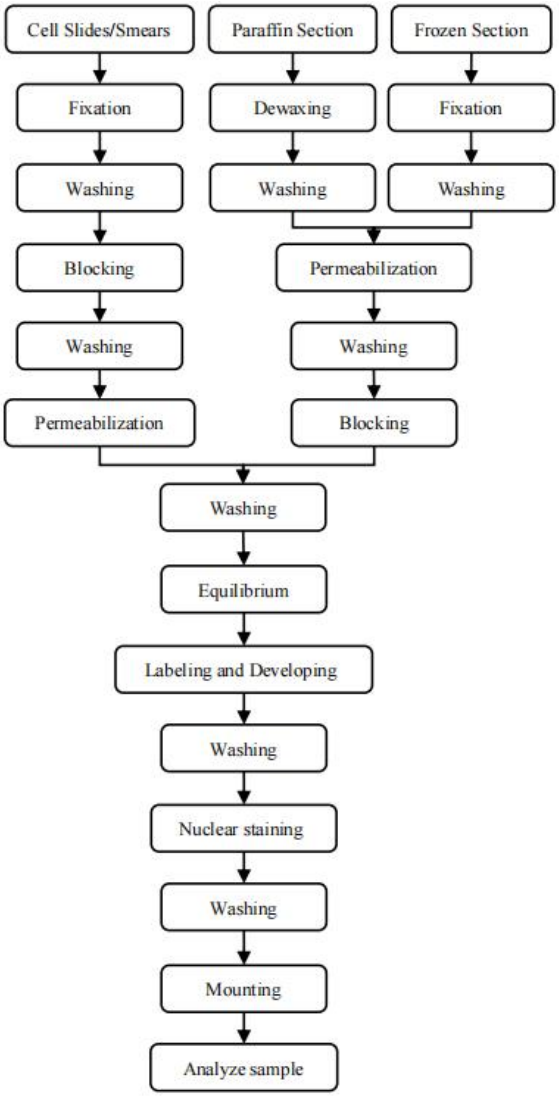
4. Other Reagents

PBS, ddH_2O, Hematoxylin, Neutral Balsam.

5. Instrument

Optical microscope.

Assay 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) **1×DAB working solution**

Dilute the DAB Concentrate (20×) with DAB Dilution Buffer to 1×DAB working solution. Prepare the fresh solution before use.

Fixation and Permeabilization

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.

Note: Cell fixation is an important step in TUNEL experiments. 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) Absorb the moisture with filter paper, immerse the slides in blocking buffer (self-prepared), and block at room temperature (15~25°C) for 10 min.

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

5) Put the slides into the Permeablilization Buffer (self-prepared), and incubate at 37°C for 10 min.

6) 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 immerse slides in absolute ethanol (self-provided) for twice, 5 min each time; 90%, 80%, 70% ethanol aqueous solution (self-provided) for once, 3 min each time.

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) Absorb the moisture around the tissue. 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.

5) Absorb the moisture around the tissue, immerse the slides in blocking buffer (self-prepared), and block at room temperature (15~25°C) for 10 min.

6) 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 experiments to confirm the incubation time.

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

5) Absorb the moisture around the tissue, immerse the slides in blocking buffer (self-prepared), and block at room temperature (15~25°C) for 10 min.

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

Labeling and developing

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 Working Solution

1) Preparation of TdT enzyme working solution

Refer to table below to prepare appropriate TdT enzyme working solution and mix well. (Prepare the fresh solution before use).

Component	Positive Control / Experimental Group	Negative Control
TdT Equilibration Buffer	40 μL	45 μL
Biotin-dUTP	5 μL	5 μL
TdT Enzyme	5 μL	0 μL
Total Volume	50 μL	50 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. 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.
3. Gently pipette the TdT enzyme Working Solution to incorporate the TdT enzyme. Stirring by vortex is not recommended.

2) **Preparation of Streptavidin-HRP working solution**

Refer to the table below to prepare appropriate Streptavidin-HRP working solution and mix well. Prepare the fresh solution before use.

Component	1 slide	5 slides	10 slides
Streptavidin-HRP	0.5 μL	2.5 μL	5 μL
PBS	99.5 μL	497.5 μL	995 μL
Total Volume	100 μL	500 μL	1000 μL

3.Labeling and developing 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 TdT enzyme working solution to each slide, and incubate at 37°C for 60 min with shading light in humidified chamber.
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 100 μL Streptavidin-HRP working solution, incubate at 37°C for 30 min with shading light in humidified chamber.
5) Wash the slide with PBS for 3 times, 5 min each time.

Note：The washing time or washing times can be appropriately extended, otherwise the residual HRP will increase the staining background.

6) Carefully blot the liquid around the sample areas with absorbent paper. Add 100 μL1×DAB working solution, incubate at RT for 30 s~5 min or incubate for appropriate time according to DAB reaction.

Note：If the color is strong, Brown can be observed under a microscope，please washing the slide with PBS immediately. If the color is weak, this step can be prolonged.

7) Wash the slide with PBS for 3 times, 5 min each time.
8) (Optional)：Add Hematoxylin staining solution to stain the nuclear, Wash the slide with PBS for 3 times, 5 min each time.

9) Wash the slide with water, then put the slides into the following reagents in order to dehydrate and permeate: 70% ethanol, 80% ethanol, 90% ethanol, anhydrous ethanolI, anhydrous ethanol II, XyleneI and Xylene II. Put the slides in each reagent for 2 min, and finally air dry the sections in the fume cupboard.
10) Drop neutral balsam (self-provided) beside the section, and cover with a coverslip, taking care to avoid air bubbles, and place the sealed sections horizontally in a fume hood to air dry.
11) Observe the dried sections and collect images with an optical microscope.

Notes

1. 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.
2. Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slides.
3. Avoid repeated freezing and thawing of the TdT enzyme. Stirring by vortex is not recommended.
4. 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.
5. 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.
6. For your safety and health, please wear a lab coat and disposable gloves during the operation.