TUNEL In Situ Apoptosis Kit (HRP-DAB Method)

Catalog No: AAA22132

Product size: 20 Assays/50 Assays/100 Assays

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
Streptavidin-HRP	10 µL	25 µL	50 µL	-20°C
Biotin-dUTP	100 µL	250 µL	500 µL	-20°C
DAB Concentrate(20 ×)	200 µL	500 µL	1 mL	-20°C
DAB Dilution Buffer	4 mL	10 mL	10 mL × 2	-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
Manual	One copy			

Introduction

TUNEL In Situ Apoptosis Kit (HRP-DAB Method) has high sensitivity and easy operation.

This kit is suitable for in situ apoptosis detection of tissue samples (paraffin sections, frozen sections), 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 biotin-labeled 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.

Detection Sample Types

☑ **Paraffin Section**

☑ **Frozen Section**

Storage

Store at -20°C, and the shelf life is one year. Streptavidin-HRP and DAB Concentrate (20×) should be stored in the dark.

Materials Not Supplied

1) Paraffin Section

Xylene, ethanol.

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

2) 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%.

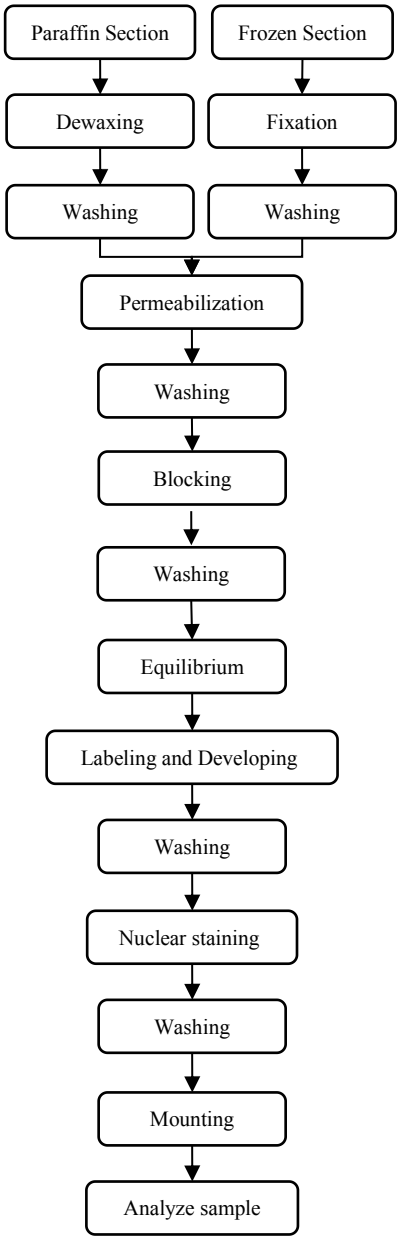
3) Other Reagents

PBS, ddH_2O, Hematoxylin, Neutral Balsam.

4) 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 ddH2O 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) **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. **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) Absorbs 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.

2. **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

1. **Group setting**

Group	Sample selection	Feature	Purpose
Positive control	Select a slice of the experimental group	Optional, DNase I treatment, cutting off DNA to produce an exposed 3 ' -OH end, as a positive sample	Verify the effectiveness of the experimental process and reagents
Negative control	Select a slice of the experimental group	Optional, label working solution does not contain TdT Enzyme	Exclude sample background and non-specific staining of samples and staining reagents.
Experimental group	Slice to be detected	It is necessary to incubate the labeled working solution to keep the consistency of experimental detection conditions.	Source of experimental data

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.

✧ **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.

✧ **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.

✧ **Experimental qroup preparation**

a) 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 blew to prepare to prepare appropriate TdT enzymeworking solution and mix well. (Prepare the fresh solution before use).

	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 before use.

	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 ethanol Ⅰ, anhydrous ethanol Ⅱ, Xylene Ⅰ 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.

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.
	Streptavidin-HRP working solution is not cleaned.	Appropriately increase the number and time of rinsing.
	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.
	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. Optimize the concentration and duration of proteinase K.
	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°C). 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.
	Inadequate intracellular hydrogen peroxide blocking results in positive staining of many cells.	Improve the blocking method of hydrogen peroxide, prolong the blocking time.
	DAB takes too long to develop color.	Properly reduce DAB color development time
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.
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.