TECHNICAL MANUAL

TUNEL 1-Step Assay Kit (HRP-DAB)

- **SKU CODE:** AKES076
- **SIZE:** 20 Assays
- **DETECTION PRINCIPLE:** Apoptosis Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Fixation and Permeabilization
- Permeabilization
- Blocking
- Equilibrium
- Labeling and Developing
- Nuclear staining
- Mounting
- Analyze sample

2. Introduction

The Assay Genie TUNEL In Situ Apoptosis Kit (HRP-DAB Method) offers 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.

3. Detection principle

When cells undergo apoptosis, specific DNA endonucleases are activated, cutting genomic DNA between 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 bind with biotin. Therefore, apoptotic cells can be observed through DAB reaction with optical microscopy.

4. Detection sample types

- Paraffin Section
- Frozen Section

5. Kit components & storage

Item	Component	20 Assays	50 Assays	100 Assays	Storage
AKES076	TdT Equilibration Buffer	5 mL	5 mL×2	5 mL×4	-20 °C
	TdT Enzyme	100 µL	250 µL	250 µL×2	-20 °C
	Proteinase K (100×)	100 µL	100 µL	100 µL	-20 °C
	Streptavidin-HRP	10 µL	25 µL	50 µL	-20 °C, shading light
	Biotin-dUTP	100 µL	250 µL	500 µL	-20 °C
	DAB Concentrate (20×)	200 µL	500 µL	1 mL	-20 °C, shading light
	DAB Dilution Buffer	4 mL	10 mL	10 mL×2	-20 °C
	DNase I (20 U/µL)	25 µL	25 µL	25 µL	-20 °C
	DNase I Buffer (10×)	1500 µL	1500 µL	1500 µL	-20 °C
	Manual	One copy	One copy	One copy	

6. Storage

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

7. Materials not supplied

1. **Paraffin Section:** Xylene, ethanol. Blocking Buffer: Dilute H2O2 with deionized water to a concentration of 3%.

2. **Frozen Section:** Fixative Buffer: Paraformaldehyde dissolved in PBS with final concentration of 4%. Blocking Buffer: Dilute H2O2 with deionized water to a concentration of 3%.
3. **Other Reagents:** PBS, ddH2O, Hematoxylin, Neutral Balsam.
4. **Instrument:** Optical microscope.

8. Reagent preparation

1. **1× Proteinase K working solution:** Add 1 µL Proteinase K (100×) to 99 µL PBS and mix well. Prepare fresh solution before use.
2. **1× DNase I Buffer:** Dilute the DNase I Buffer (10×) with ddH2O to 1× DNase I buffer. Prepare 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 fresh solution before use. Note: Do not vortex the DNase I as it 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 fresh solution before use.

9. Fixation and permeabilization

Paraffin section

1) Deparaffinize and hydrate the paraffin slides by conventional methods. Immerse slides in xylene (self-prepared) twice for 10 min each time, then immerse slides in absolute ethanol (self-provided) twice for 5 min each time; 90%, 80%, 70% ethanol aqueous solution (self-provided) once for 3 min each time.

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

2) Wash the slides with PBS 3 times, 5 min 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 incubation time for samples from different tissues or species may vary. It is recommended to perform a preliminary experiment to confirm the incubation time.

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

2. Frozen section

1) Take out the frozen sections, equilibrate 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 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 incubation time for samples from different tissues or species may vary. It is recommended to perform preliminary experiments to confirm the incubation time.

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

10. Labeling

Group setting

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

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 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 3 times, 5 min each time.

Experimental group preparation

a) After the experimental group completed the permeabilization 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 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 dissolves and mix fully before use. It is a normal phenomenon that TdT Equilibration Buffer crystallizes 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

11. 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 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 3 times, 5 min each time. Note: The washing time or washing frequency can be appropriately extended, otherwise residual HRP will increase the staining background.

6. Carefully blot the liquid around the sample areas with absorbent paper. Add 100 µL 1× 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 wash the slide with PBS immediately. If the color is weak, this step can be prolonged.

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

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

9. Wash the slide with water, then place 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. Place 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.

12. 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.
Non-specific staining	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.
Non-specific staining	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.
Non-specific staining	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.
Non-specific staining	Inappropriate fixatives are used, such as acidic fixatives.	Use recommended Fixative Buffer.
Non-specific staining	Streptavidin-HRP working solution is not cleaned.	Appropriately increase the number and time of rinsing.
Non-specific staining	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.
Little or poor staining	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.
Little or poor staining	Insufficient deparaffinization of Paraffin section.	Extend dewaxing time or replace with a new dewaxing solution.

Symptoms	Causes	Comments
Little or poor staining	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.
Little or poor staining	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.
High background	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.
High background	Inadequate intracellular hydrogen peroxide blocking results in positive staining of many cells.	Improve the blocking method of hydrogen peroxide, prolong the blocking time.
High background	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.
Positive control has no signal	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.

13. 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) and 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.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.