Technical Manual

TUNEL 1-Step In Situ Apoptosis Kit

- **Catalogue Code:**
  - **AKES071**
  - **AKES072**
  - **AKES073**
  - **AKES074**
  - **AKES075**
- **Size: 20/50/100 Assays**
- **Research Use Only**

1. Description and Principle

The Assay Genie TUNEL 1-Step In Situ Apoptosis Kit is a highly sensitive, fast and simple method to detect apoptosis. This kit is suitable for in situ apoptosis detection of tissue samples (paraffin or frozen sections) and cells (cell slides and cell smears). The results can be directly detected with a fluorescence microscope.

How Do Our 1-Step TUNEL assays Work?

When cells undergo apoptosis, specific DNA endonucleases are activated, causing breaks in the genomic DNA between nucleosomes. Apoptotic cell DNA is cleaved into multimers of 180~200bp fragments, corresponding to the oligonucleosomal size. The DNA of apoptotic cells typically migrates as a ladder of approximately 180 - 200bp on an agarose gel. The exposed 3'-OH of the broken DNA can tagged with a fluorescently labelled dUTP all catalysed by Terminal Deoxynucleotidyl Transferase (TdT). The fluorescently labelled DNA can be detected with a fluorescence microscope.

2. Key features and Sample Types

Detection Method: Fluorometric

Sample Type: Cell Sample, Paraffin Sections, Frozen Section

Applications: Apoptosis/DNA Fragmentation.

3. Kit Contents

Product	Size	Cat. Code
TUNEL 1-Step In Situ Apoptosis Kit	20/50/100 Assays	AKES071 AKES072 AKES073 AKES074 AKES075

Each kit contains reagents for 20/50/100 assays including:

Item	20 Assays	50 Assays	100 Assays	Storage
Dewaxing Solution (10 x)	20 mL	50 mL	100 mL	RT
TdT Equilibration Buffer	4 mL	9 mL	9 mL x 2	-20˚C
TdT Enzyme	100 µL	250 µL	250 µL x 2	-20˚C
Proteinase K (100 x)	20 µL	50 µL	100 µL	-20˚C
Labelling Solution AKES071 (FITC) AKES072 (FL488) AKES073 (FL594) AKES074 (FL647) AKES075 (FL555)	100 µL x 2	100 µL x 5	100 µL x 10	-20˚C
DNase I (20 U/µL)	5 µL	13 µL	25 µL	-20˚C
DNase I Buffer (10 x)	300 µL	700 µL	1500 µL	-20˚C
DAPI (25 x)	100 µL	250 µL	500 µL	-20˚C
Product Description	One Size			

Additional Materials required

1. Cell Sample
    a. Fixative buffer: Polyformaldehyde dissolved in PBS with a final concentration of 4%.
    b. Permeabilization Buffer: Triton-100 dissolved in PBS with a final concentration of 0.2%. The prepared solution can be used after storage at 4°C for 1~2 days.

2. Frozen Section
    a. Fixative buffer: Polyformaldehyde dissolved in PBS with a final concentration of 4%.

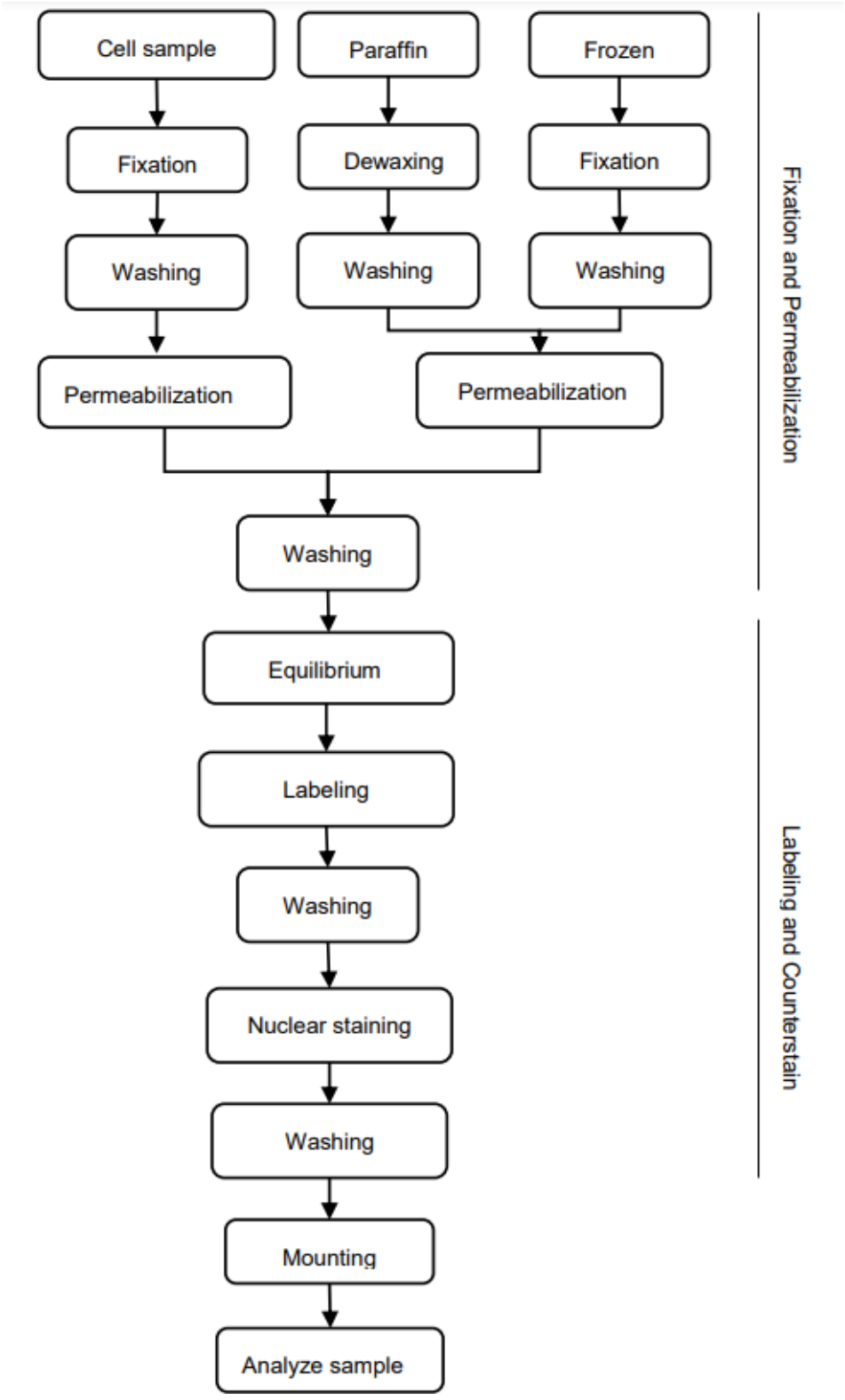
3. PBS, ddH_2O, anti-fluorescence quenching agent.

4. Fluorescence microscope.

4. Shipping and Storage

Store Dewaxing solution at room temperature. Store all other reagents at -20°C for 12 months. The labeling Solution and DAPI (25 ×) should be stored in the dark.

5. Assay Procedure



6. Pre-assay Preparation

Reagent Preparation

1. **Dewaxing Solution (1X) (Specific for Paraffin Sections)**
   Dilute the Dewaxing Solution (10x) with ddH_2O at the ratio of 1:9

Volume of Dewaxing Solution (1×)	Number of samples (PCS)
200 mL	≤24
500 mL	≤60
1000 mL	≤120

Note:

a. Before use, heat the diluted solution at 60°C in an oven for 30 min, then mix fully.
b. It is suggested to dilute the dewaxing solution before use.
c. If the dewaxing solution needs to be reused, use it within a week. Do not reuse the solution more than 3 times. For each re-use, extend the dewaxing time by 5～10 min.

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

3. **DNase I Buffer (1X)**
   Dilute the DNase I Buffer (10 ×) with ddH_2O to 1 × DNase I buffer. Prepare fresh solution before use.

4. **DNase I working solution (200 U/mL)**
   Dilute the DNase I (20 U/µL) with 1 × DNase I buffer to form a 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.

5. **DAPI working solution**
   Add 4 µL DAPI (25 ×) to 96 µL PBS and mix well. Prepare fresh solution beforeuse.

Fixation and Permeabilization

1. **Cell sample**
   a) Immerse the naturally dried cell slides or smears into fixative buffer (self-prepared) at RT for 15~20 min (or at 4°C for 1~2 h).
   Note: Cell fixation is an important step in analysing apoptotic samples. Unfixed cells may lose smaller DNA fragments, leading to lower signals.

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

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

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

2. Paraffin Section

a) Dewax the slides with 1×Dewaxing Solution. Reheat the dewaxing solution at 60°C for 30 min, immerse the slides into the dewaxing solution and incubate at 60°C for 30 min.
Note:
- Low temperatures may affect dewaxing. It is recommended to bring the dewaxing solution to 60°C for 30 min before use. If the paraffin section is thick, the dewaxing time can be extended to 50 min.
- Avoid direct alignment with the air outlet of the oven to avoid false positives of DNA fragmentation due to high temperatures.

b) Wash the slides with tap water for 5 min until froth free.
Note: Don't wash the tissue directly, avoid tissue damage.

c) Absorb the moisture around the tissue with filter paper. Or immerse the slides in PBS.

d) 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 perform a preliminary experiment to confirm the incubation time.

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

3. Frozen Section

a) Immerse the frozen slides in Fixative Buffer (self-prepared), and incubate at RT (15~25°C) for 30 min.

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

c) 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 perform a preliminary experiment to confirm the incubation time.

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

7. Experimental Operation

Labelling

Preparation Procedure

1. **(Optional) Preparation of Positive and Negative Controls**
   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 for each experiment.
   Note: The preparation of positive and negative controls can be performed at the same time.

   a) Positive Control
   - Add 100 µL of 1 × DNase I Buffer to each slide, and incubate at RT for 5 min.
   - Carefully blot the liquid around the sample areas with absorbent paper. Add 100 µL DNase I working solution (200 U/mL) to each slide, and incubate at 37°C for 10~30 min.
   - Wash the slide with PBS 3 times, 5 min each time.

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

2. **Preparation of Labelling Working Solution**
   Prepare the Labelling Working Solution according to the number of samples. Please refer to the table below (prepare fresh solution before use).

Component	Positive Control/ Experimental group	Negative Control
TdT Equilibration Buffer	35 µL	40 µL
Labelling Solution	10 µL	10 µL
TdT Enzyme	5 µL	0 µL

Note:

a. Bring the TdT Equilibration Buffer to RT until the liquid is completely dissolved and mix fully before use. It's normal that TdT Equilibration Buffer may crystallize after melting.
b. Before using the Labeling Solution, dissolve on ice and mix fully.
c. TdT Enzyme is temperature sensitive, store it strictly at -20°C.
d. Gently pipette the Labelling Working Solution to incorporate the TdT enzyme. Stirring by vortex is not recommended.

Labelling 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 Labelling working solution to each slide, and incubate at 37°C for 60 min with shading light in a humidified chamber.
   Note: If signal intensity is low, the incubation time for the DNA-labelling reaction can be extended. Labelling times of up to 4 hours at 37°C may be required for some systems.

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 DAPI working solution, and incubate at RT for 5 min with shading light.

5. Wash the slides with PBS 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.

8. Analysis

SKU	Dye	Ex/Em (nm)	Filter Set
AKES071	FITC	490/520	FITC Filter Set
AKES072	FL488	495/519	FITC Filter Set
AKES073	FL594	590/617	TRITC Filter Set
AKES074	FL647	650/665	Cy5 Filter Set
AKES075	FL555	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.

9. Important General Notes:

Problem	Causes	Solutions
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 Tdtenzyme 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 pH 7.4
	Fixing time is too long, resulting in high degree of cross-linking	Reduce fixation time, or fix with 2% paraformaldehyde dissolved in PBS pH 7.4
	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°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
	The autofluorescence caused by haemoglobin in red blood cells causes serious interference cells that divide and proliferate at a high speed and have DNA breaks in the nucleus	Other apoptosis detection kits can be used
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 or extend washing time
	For cell samples, 0.2% Triton-100 does 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

Additional Notes

1. This kit is for research use only.

2. Please take note of 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.

5. Avoid repeated freezing and thawing of the Labelling Solution and TdT enzyme. Mixing 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 thus select the most suitable experimental conditions.

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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.

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