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One-step TUNEL Assay Kit

(Red, AF647)

Catalog No.: E-CK-A324

Manual

FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS

Elabscience®

Focus on Your Research Service for Life Science

（Please read this manual carefully before use. If you have any questions, please contact us in time.）

Introduction

Elabscience® One-step TUNEL Assay Kit apply a highly sensitive, fast and simple method to detect cell apoptosis. This kit is suitable for in situ apoptosis detection of tissue samples (Paraffin embedding, frozen section) and cells (Cell smears, cell climbing smears) in situ apoptotic detection. The test 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. After extraction and loading for electrophoresis, a DNA ladder of 180-200bp can be found in such DNA samples, which is a specific event during cell apoptosis. 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 microscopy or flow cytometer.

Sample Types

☑ Cell Sample ☑ Paraffin Section ☑ Frozen Section

Components

Cat.	Products	Cap Code	20 Assays	50 Assays	100 Assays
E-CK-A32A	TdT Equilibration Buffer		4 mL	9 mL	9 mL × 2
E-CK-A32B	TdT Enzyme		100 μL	250 μL	250 μL × 2
E-CK-A32C	Proteinase K (100 ×)		20 μL	50 μL	100 μL
E-CK-A324D	Labeling Solution		100 μL × 2	100 μL × 5	100 μL × 10
E-CK-A32E	DNase I (2 U/μL)		5 μL	13 μL	25 μL
E-CK-A32F	DNase I Buffer (10 ×)		100 μL	250 μL	500 μL
E-CK-A32G	DAPI (25 ×)		100μL	250μL	500μL
Manual	One Copy				

Storage

Store at -20 ℃ for 12 months. Labeling Solution and DAPI (25 ×) should be stored in the dark.

Reagent Not Included

1. Cell Sample: Fixative Buffer (Polyformaldehyde dissolved in PBS with final concentration of 4%). Permeablilization Buffer (Triton-100 dissolved in PBS with final concentration of 0.2%).

2. Paraffin Section： Xylene, ethanol, PBS.

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 microscopy

Reagent Preparation

a) **1 × Proteinase K working solution：** Ready to use as supplied. Add 1 μL Proteinase K (100 ×) to 99 μL PBS and mix well.

b) **1 × DNase I Buffer：** Ready to use as supplied. Dilute the DNase I Buffer (10 ×) with ddH_2O to 1 × DNase I buffer.

c) **DNase I working solution（20 U/mL）：** Ready to use as supplied. Dilute the DNase I (2 U/μL) with 1 × DNase I buffer (Reagent preparation b) to DNase I working solution (20 U/mL).

d) **DAPI working solution：** Ready to use as supplied. Add 4 μL DAPI (25 ×) to 96 μL PBS and mix well.

e) **Labeling Working Solution：** Calculate the sample volume and configure it centrally. The amount of each sample is prepared according to the following table. Mix well and ready to use as supplied.

Component	Positive Control / experimental	Negative Control
TdT Equilibration Buffer	35 μl	40 μl
Labeling Solution	10 μl	10 μl
TdT Enzyme	5 μl	0 μl

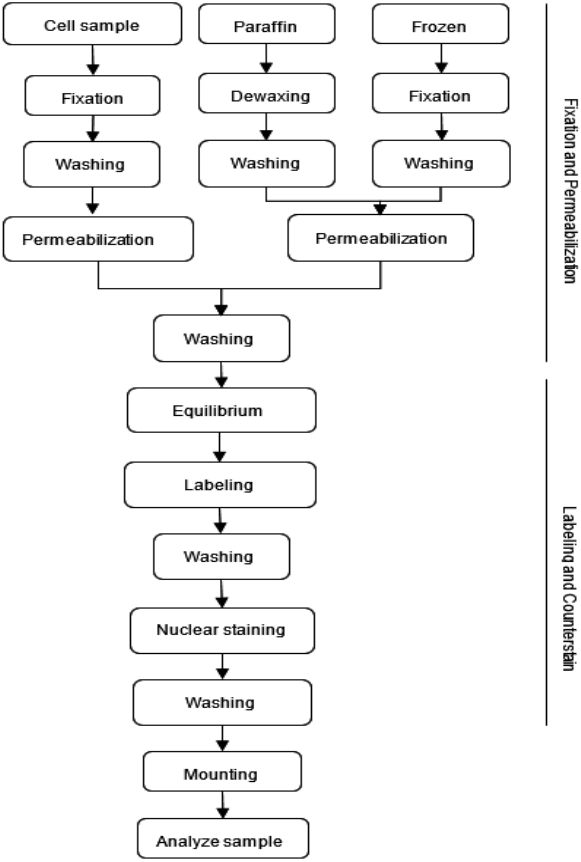
Note: 1. Bring the TdT Equilibration Buffer to room temperature until the liquid completely dissolved. It is normal for the frozen TdT Equilibriation Buffer to show cobalt salt crystals after melting. Please mix fully before use.

2. Before using the Labeling Solution, please dissolve it on ice. After it is completely dissolved, centrifuge it and mix it by pipetting. TdT Enzyme is sensitive to temperature, please store it strictly at -20℃, take it out before use, and put it back immediately after use.

3. When preparing the Labeling Working Solution, the time of vortex should not be too long.

4. Do not vortex the DNase I solution as DNase I will denature with vigorous mixing.

Experimental Procedure



Fixation and Permeabilization

The positive control samples need to be treated with DNase I enzyme after permeabilization. For specific operation steps, see the **Positive and Negative Control Sample Preparation** on the next page.

- **Cell sample**

1）Immerse the naturally dried cell slide or smear into the fixative, 4℃ for 25 min.

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

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

3）Put the slides into the Permeablilization Buffer, and incubate at 37℃ for 10 min.

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

- **Paraffin section**

1）Deparaffinize and hydrate the paraffin slides by conventional methods. Immerse slides in Xylene 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.

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

3）Add 100 μL of 1 × Proteinase K working solution (Reagent preparation a) to each sample, and incubate at RT for 20 min.

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

➢ **Frozen section**

1）Immerse the frozen sections in the Fixative Buffer, and incubate at RT (15~25 ℃) for 30 min.

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

3）Add 100 μL of 1 × Proteinase K working solution (Reagent preparation a) to each sample, and incubate at RT for 10 min.

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

Labeling and Staining

1）Add 100 μL of TdT Equilibration Buffer to each sample, and incubate at RT 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 Labeling working solution (Reagent preparation e) to each slide, and incubate at 37 ℃ for 60 min 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 ℃ may be required for some systems.

3）Wash the slide 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 (Reagent preparation d), and incubate at RT for 5 min.

5）Wash the slide 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 to seal the slides.

Analyze Sample

Immediately analyze samples under a fluorescence microscope using a standard fluorescein filter set to view the red fluorescence of AF647 at 665 ±20nm; and blue fluorescence of DAPI at 460nm.

Note：Observe slides as soon as possible. If it cannot be observed immediately, please store at 4 ℃ and protect from light.

Positive and Negative Control Sample Preparation

Positive and negative controls should be set up to show the objectivity and accuracy of TUNEL. The control samples should be prepared according to the following operations, and the remaining steps should be carried out in the same way as the samples to be tested.

- **Positive control preparation**

Use DNase I to process the positive control sample according to the following steps, and the rest of the steps are the same as the samples to be tested.

1）Add 100 μL 1 × DNase I Buffer (Reagent preparation b) to each slide, and incubate at 37 ℃ for 5 min.

2）Carefully blot the liquid around the sample areas with absorbent paper. Add 100 μL DNase I working solution (20 U/mL) (Reagent preparation c) on each

slide, and incubate at RT for 10~30 min.

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

- **Negative control preparation**

The Labeling Working Solution (Reagent preparation e) does not add TdT enzyme, and the rest of the steps are the same as the experimental group.

Cautions

1. Avoid repeated freezing and thawing of the Labeling Solution and TdT enzyme, Vortex and mix fully before use.

2. After washing the slides with PBS, please carefully blot the liquid around the sample areas with absorbent paper.

3. Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slices.

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 kit is for research use only.

6. Please take safety precautions and follow the procedures of laboratory reagent operation.

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°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 hemoglobin in red blood cells causes serious interference Cells that divide and proliferate at a high speed sometimes have DNA breaks in the nucleus	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
Loss of sample from the slides	The sample is digested by the enzyme from the slide	Reduce the processing time of proteinase K

This manual must be read attentively and completely before using this product.
If you have any problems, please contact our Technical Service Center for help.
Phone: 240-252-7368(USA)
Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.