Elabscience®

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TUNEL Assay Kit (Enhanced TMR Red)

Catalog No: E-CK-A335

Sizes： 20 Assays / 50 Assays / 100 Assays

Cat.	Products	20 Assays	50 Assays	100 Assays	Storage
E-CK-A335A	TdT Equilibration Buffer (5 ×)	700 μL	900 μL × 2	3.6 mL	-20°C
E-CK-A335B	TdT Enzyme	20 μL	50 μL	100 μL	-20°C
E-CK-A335C	Proteinase K (100 ×)	20 μL	50 μL	100 μL	-20°C
E-CK-A335D	Enhanced Fluorescein Mix	100 μL	250 μL	500 μL	-20°C
E-CK-A335E	DNase I (2 U/μL)	5 μL	13 μL	25 μL	-20°C
E-CK-A335F	DNase I Buffer(10 ×)	100 μL	250 μL	500 μL	-20°C
Manual			**One Copy**		

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.

Introduction

Elabscience® TUNEL Apoptosis Assay Kit is a highly sensitive, rapid and simple method for cell apoptosis detection. The result can be detected by fluorescence microscopy.

This kit is developed to detect tissue (Paraffin embedding, freezing and ultrathin section) and cells (Cell smears, cell climbing smears) in situ apoptotic detection.

Detection principle

When cells undergo apoptosis, endonuclease enzymes are activated to cleave the genomic DNA between nucleosomes. When genomic DNA is cleaved, the exposed 3'-OH can be added with Tetramethylrhodamine-deoxyuridine triphosphate (TMR Red-dUTP) due to the catalysis of Terminal Deoxynucleotidyl Transferase (TdT), apoptosis cells can be detected by fluorescence microscopy. This is the principle of TUNEL (TdT-mediated dUTP Nick-End Labeling) for detecting apoptosis.

The Enhanced Fluorescein Mix [E-CK-A335D] in the kit contains TMR Red and fluorescence enhancement factor. The fluorescence enhancement factor can bind to TMR Red and enhance the stability amplifies of the signal, which makes the fluorescence brighter and has stronger anti-quenching ability.

Reagent not included

1. Cell Sample

Fixative Buffer (Polyformaldehyde dissolved in PBS with final concentration of 4%)(It is recommended to use Elabscience® E-IR-R113).

Permeablilization Buffer (0.1% Triton-100, It is recommended to use Elabscience® E-IR-R122)

2. Paraffin Embedding

Xylene, ethanol, PBS (It is recommended to use Elabscience® E-IR-R187).

3. Freezing Section

Fixative Buffer (Polyformaldehyde dissolved in PBS with final concentration of 4%)(It is recommended to use Elabscience® E-IR-R113).

4. Other Reagents

PBS, ddH2O, DAPI (It is recommended to use Elabscience® E-IR-R103), Anti Fluorescence Quenching Agent (It is recommended to use Elabscience® E-IR-R119).

Instructions

Proteinase K (100 ×) [E-CK-A335C] is concentrated, diluted with PBS to 1 × working solution before use.

For example: take 1 μL Proteinase K (100 ×) [E-CK-A335C], add to 99 μL PBS, the mixture is 1 ×Proteinase K working solution.

Experimental Procedure

The sample preparation of TUNEL is the key to the experiment. The conditions recommended in this manual are universal. Users need to adjust the experimental conditions according to their own sample materials and the pre-experimental results, such as processing time, concentration and so on to optimize the suitable experimental conditions for the sample.

1. Sample Preparation

A. Adherent Cells or Cell Smears

1) Wash cells once with PBS, if the cells are not well attached, the sample can be dried to make the cells stick more firmly.
2) Fix cells in Fixative Buffer at RT (15~25 ℃) for 15~60 min.
   (Fixative Buffer: Polyformaldehyde dissolved in PBS with final concentration of 4%, prepare before use)
3) Wash cells with PBS for 3 times, 5 min each time.
4) Wipe dry the liquid around the tissue on the slides with absorbent paper. Add 1×Proteinase K working solution on the slides, incubate at 20~37 ℃ for 15~30 min or Put the slides into the Permeablilization Buffer on ice bath for 2 min.
   (1×Proteinase K working solution: Take 1 μL Proteinase K (100 ×) [E-CK-A335C], add to 99 μL PBS)
   (Permeablilization Buffer: Triton-100 dissolved 0.1% citrate sodium with final concentration of 0.1%, prepare before use)
   Tip: It's recommended that using Triton-100 penetrating Buffer to avoid dislodging caused by penetration.
5) Wash cells with PBS for 3 times, 5 min each time.
   Tips:
   To prevent the sample from falling off, please use silane or polylysine treated slide.
   Fixed samples can be immersed in 70% ethanol at -20 ℃ for 30 min or overnight to improve cell permeability.
   When wash the slides with PBS, do not add PBS directly to the cell samples, avoid the cells falling off.
   Fixative buffer, PBS, Permeablilization Buffer are not included in this kit.

B. Paraffin section

1) Dewax and hydrate the paraffin slices by conventional methods.
   (For example: Immerse slices in Xylene dewaxing twice, 5~10 min each time, then hydrate the paraffin sections with ethanol hydrated (dewaxed sections were put into 100% ethanol, 95% ethanol, 90% ethanol, 80% ethanol, 70% ethanol, 2 min each step).
2) Wash the slide with PBS for 3 times, 5 min each time.
3) Wipe dry the liquid around the tissue on the slides with absorbent paper. Add 1×Proteinase K working solution on the slides, incubate at 20~37 ℃ for 15~30 min.
   (1×Proteinase K working solution: Take 1 μL Proteinase K (100 ×) [E-CK-A335C], add to 99 μL PBS)

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

Tip：Since the Concentration, time and temperature of protease K treatment may vary according to different types of tissue, which needs to be determined by the end user after exploring the conditions.

C. Frozen Section

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

(Fixative Buffer: Polyformaldehyde dissolved in PBS with final concentration of 4%, prepare before use)

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

3) Wipe dry the liquid around the tissue on the slides with absorbent paper. Wipe dry the liquid around the tissue on the slides with absorbent paper. Add 1 ×Proteinase K working solution on the slides, incubate at 20~37 ℃ for 15~30 min.

(1 ×Proteinase K working solution: Take 1 μL Proteinase K (100 ×) [E-CK-A335C], add to 99 μL PBS)

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

D. 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 methods, and the remaining steps should be carried out in the same way as the samples to be tested.

1) Positive Control Preparation

Samples were treated according to the experimental steps mentioned above. Then add 100 μL DNase I working solution on the slide and incubate at RT (25~37 ℃) for 10-30 min. The remaining steps are the same as tests slides.

a) Dilute the DNase I Buffer (10 ×) [E-CK-A335F] with ddH_2O to 1 × DNase I buffer.

b) Add 100 μL 1 × DNase I buffer to each slide, incubate at RT for 5 min.

c) Dilute the DNase I concentrate (2 U/μL) [E-CK-A335E] with 1 × DNase I buffer to DNase I working solution(20 U/mL).

d) Wipe dry the liquid around the tissue on the slides with absorbent paper. Add 100 μL DNase I working solution(20 U/mL) on each slide, incubate at RT for 10~30 min.

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

Tip: In order to avoid false positive signals in the experimental group caused by residual DNase I on the slide, a separate staining cylinder must be used for the positive sample control slide.

2) Negative Control Preparation

Do not add TdT Enzyme during the labeling reaction (TdT Enzyme working solution) in the following experimental procedures, and the other steps are the same as tests slides.

2. Experimental Procedure of TUNEL

A. Reagents Preparation

TdT Enzyme Working Solution Preparation

Refer to the table below to prepare appropriate TdT Enzyme working solution (according to actual needs), prepare before use.

	1 slide	5 slides	10 slides
ddH_2O	34 μl	170 μl	340 μl
TdT Equilibration Buffer (5 ×)	10 μl	50 μl	100 μl
Enhanced Fluorescein Mix	5 μl	25 μl	50 μl
TdT Enzyme	1 μl	5 μl	10 μl
Total Volume	50 μl	250 μl	500 μl

B. Procedure of TUNEL(Detect by Fluorescence Microscopy)

1. Dilute the TdT Equilibration Buffer (5×) [E-CK-A335] with ddH_2O to 1 × TdT Equilibration working buffer. Add 100 μL 1 × TdT Equilibration working buffer to each slide and incubate at RT for 30 min.
2. Wipe dry the liquid around the tissue on the slides with absorbent paper.Add 50 μL TdT Enzyme working solution preparation to each slide, incubate at 37℃ for 60 min in wet bow(Do not add TdT Enzyme into the TdT Enzyme working solution with negative control).
3. Wash the slide with PBS for 3 times, 5 min each time.
4. Wipe dry the liquid around the tissue on the slides with absorbent paper. Add DAPI, incubate at RT for 5 min.
5. Wash the slide with PBS for 4 times, 5 min each time. Wipe dry the liquid around the tissue on the slides with absorbent paper. Add mounting medium (contain Fluorescent Mounting Media) to seal the slides.
6. Observe the dried sections and collect images with a fluorescence microscopy.

Storage

Store at -20 ℃. Enhanced Fluorescein Mix [E-CK-A335D] should be stored in dark.

Cautions

1. For maximal assay performance, this reagent should be used within 12 months. Avoid freeze / thaw cycles.
2. After washing the slides with PBS, please wipe dry the liquid around the tissue on the slides with absorbent paper.
3. Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slices.
4. Prepare the TdT Enzyme working solution before use, it can be store on ice for short time, enzyme activity may lose after long-term preservation.
5. This kit is for research use only. For your safety and health, please wear lab clothes and gloves. Instructions should be followed strictly, changes of operation may result in unreliable results.