SZABO SCANDIC

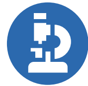
Part of Europa Biosite

Produktinformation

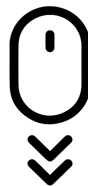
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien
T. +43(0)1 489 3961-0
F. +43(0)1 489 3961-7
mail@szabo-scandic.com
www.szabo-scandic.com

Elabscience®

8th Edition, revised in May, 2020

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

TUNEL Apoptosis Assay Kit (HRP-DAB)

Catalog No: E-CK-A331

Sizes: 20 Assays / 50 Assays / 100 Assays

Cat.	Products	20 Assays	50 Assays	100 Assays	Storage
E-CK-A331A	TdT Equilibration Buffer	1.0 mL	1.25 mLx 2	5.0 mL	-20 ℃
E-CK-A331B	TdT Enzyme	80 μL	200 μL	400 μL	-20 ℃
E-CK-A331C	Proteinase K (50 ×)	40 μL	100 μL	200 μL	-20 ℃
E-CK-A331D	Streptavidin-HRP	10 μL	25 μL	50 μL	2~8 ℃
E-CK-A331E	Biotin-dUTP	20 μL	50 μL	100 μL	-20 ℃
E-CK-A331F	DAB Concentrate (20 ×)	200 μL	500 μL	1 mL	-20 ℃
E-CK-A331G	DAB Dilution Buffer	4 mL	10 mL	10 mL×2	-20 ℃
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.

Copyright ©2020-2021 Elabscience Biotechnology Inc. All Rights Reserved

Introduction

Elabscience® TUNEL Apoptosis Assay Kit is a highly sensitive, rapid and simple method for cell apoptosis detection. After Biotin-dUTP labeling and DAB staining, cell apoptosis can be detected under ordinary optical 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 a biotinylated dUTP (Biotin-dUTP) due to the catalysis of Terminal Deoxynucleotidyl Transferase (TdT), followed by the binding of horseradish Peroxidase-labeled Streptavidin (Streptavidin-HRP). Thereafter, through the HRP catalysis, the apoptotic cells are marked by DAB, which can be detected by ordinary microscopy. This is the principle of TUNEL (TdT-mediated dUTP Nick-End Labeling) for detecting apoptosis.

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).

   Blocking Buffer (H_2O_2 dissolved in Deionized water with final concentration of 3%)(It is recommended to use Elabscience® E-IR-R115).

   Permeablilization Buffer (Triton-100 dissolved in 0.1% citrate sodium with final concentration of 0.1%)(It is recommended to use Elabscience® E-IR-R122).

2. **Paraffin Embedding**

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

   Blocking Buffer (H_2O_2 dissolved in Deionized water with final concentration of 3%)(It is recommended to use Elabscience® E-IR-R115).

3. **Freezing Section**

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

   Blocking Buffer (H_2O_2 dissolved in Deionized water with final concentration of 3%)(It is recommended to use Elabscience® E-IR-R115).

   Permeablilization Buffer (Triton-100 dissolved in 0.1% citrate sodium with final concentration of 0.1%)(It is recommended to use Elabscience® E-IR-R122).

4. **Positive Control**

   DNase I

5. **Other Reagents**

   PBS(It is recommended to use Elabscience® E-IR-R187), Hematoxylin (nuclear staining buffer, It is recommended to use Elabscience® E-IR-R120), Neutral Balsam(It is recommended to use Elabscience® E-

Copyright ©2020-2021 Elabscience Biotechnology Inc. All Rights Reserved

IR-R118).

Instructions

1. Proteinase K (50 ×) [E-CK-A331C] is concentrated, diluted with PBS to 1 ×working solution before use. For example: take 10 μL Proteinase K (50 ×) [E-CK-A331C], add to 490 μL PBS, the mixture is 1× Proteinase K working solution.
2. DAB Concentrate (20 ×) [E-CK-A331F] is concentrated, diluted with DAB Dilution Buffer [E-CK-A331G] to 1 ×working solution before use.
   For example: take 10 μL DAB Concentrate (20 ×)[E-CK-A331F], add to 190 μL DAB Dilution Buffer [E-CK-A331G], the mixture is 1×DAB 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 Blocking buffer on the slides, and incubate at RT (15~25 ℃) for 10 min.
   (Blocking Buffer: H_2O_2 dissolved in Deionized water with final concentration of 3%)
   5） Wash cells with PBS for 3 times, 5 min each time.
   6） Wipe dry the liquid around the tissue on the slides with absorbent paper. Put the slides into the Permeablilization Buffer on ice bath for 2 min.
   (Permeablilization Buffer: Triton-100 dissolved 0.1% citrate sodium with final concentration of 0.1%, prepare before use)
   7） Wash cells with PBS for 3 times, 5 min each time.

   TIPs：
   1） To prevent the sample from falling off, please use silane or polylysine treated slide.
   2） Fixed samples can be immersed in 70% ethanol at -20 ℃ for 30 min or overnight to improve cell permeability.
   3） When wash the slides with PBS, do not add PBS directly to the cell samples, avoid the cells falling off.
   4） Fixative buffer, PBS, sealing fluid, Permeablilization Buffer are not included in this kit.

Copyright ©2020-2021 Elabscience Biotechnology Inc. All Rights Reserved

B. Paraffin Section

1）Dewax and hydrate the paraffin slices by conventional methods.

(For example：Samples immerse 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 2 μL Proteinase K (50 ×)[E-CK-A331C], add to 98 μL PBS)

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

5）Wipe dry the liquid around the tissue on the slides with absorbent paper. Add Blocking Buffer on the slides, incubate at RT (15~25 ℃) for 10min.

(Blocking Buffer: H_2O_2 dissolved in Deionized water with final concentration of 3%)

6）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. Add Blocking Buffer on the slides, incubate at RT (15~25 ℃) for 10min.

(Blocking Buffer: H_2O_2 dissolved in Deionized water with final concentration of 3%)

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

5) Wipe dry the liquid around the tissue on the slides with absorbent paper. Put the slides into the Permeablilization Buffer on ice bath for 2 min.

(Permeablilization Buffer: Triton-100 dissolved 0.1% citrate sodium with final concentration of 0.1%, prepare before use)

6) 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.

Copyright ©2020-2021 Elabscience Biotechnology Inc. All Rights Reserved

1) Positive Control Preparation

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

(DNase I working Buffer：10 U-3000 U DNase I, 40 mM Tris-HCl pH 7.9, 10 mM NaCl, 6 mM $MgCl_2$, 10 mM $CaCl_2$)

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

a) 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
TdT Equilibration Buffer	45 μl	225 μl	450 μl
Biotin-dUTP	1 μl	5 μl	10 μl
TdT enzyme	4 μl	20 μl	40 μl
Total volume	50 μl	250 μl	500 μl

b) Streptavidin-HRP Working Solution

Refer to the table below to prepare appropriate Streptavidin-HRP working solution, 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

c) 1×DAB Working Solution

Refer to the table below to prepare appropriate DAB working solution, prepare before use.

	1 slide	5 slides	10 slides
20×DAB(10 mg/ml)	5 μl	25 μl	50 μl
DAB Dilution Buffer	95 μl	475 μl	950 μl
Total volume	100 μl	500 μl	1000 μl

B、Procedure of TUNEL

1）Wash the slide with PBS for 2 times, 5 min each time. Wipe dry the liquid around the tissue on the slides with absorbent paper.

2）Add 50 μL TdT Enzyme working solution preparation to each slide, incubate at 37 ℃ for 60 min

Copyright ©2020-2021 Elabscience Biotechnology Inc. All Rights Reserved

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 50 μL Streptavidin-HRP working solution, incubate at 37 ℃ for 30 min in wet bow.

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

6) Wipe dry the liquid around the tissue on the slides with absorbent paper. Add 50~100 μL DAB working solution, incubate at RT for 30 s~5 min or incubate for appropriate time according to pornographic conditions.

Tip：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 or even incubate overnight.

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, 95% 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 beside the section, and then cover them with the cover glass. In order to avoid air bubble, firstly lay one side of the cover glass flat and then gently lay another side flat. Finally dry the sealed sections by laying them in the fume cupboard.

11) Observe the dried sections and collect images with a microscope.

Storage

Streptavidin-HRP[E-CK-A331D] should be stored at 2~8 ℃, other reagents can be stored at -20 ℃.

Streptavidin-HRP[E-CK-A331D] and 20×DAB Concentrate [E-CK-A331F] 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. If the 20×DAB Concentrate became dark purple, please do not use it.
6. 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.

Copyright ©2020-2021 Elabscience Biotechnology Inc. All Rights Reserved