Product Information

YF®594 TUNEL Assay Apoptosis Detection Kit

Catalog Number: T6014

Product Size: 20 T, 50 T

Contents:

Component	20 T	50 T
A. TUNEL Equilibration Buffer	2×1 mL	5 mL
B. YF®594 TUNEL Reaction Buffer	2×0.5 mL	5×0.5 mL
C. TdT Enzyme	20 μL	50 μL
D. Proteinase K (2 mg/mL)	40 μL	100 μL
E. DNase I (2 U/μL)	5 μL	13 μL
F. 10 × DNase I Buffer	100 μL	260 μL

Storage

This product should be stored at -20 ℃ ; TUNEL Reaction Buffer should be stored in dark at -20 ℃ and avoid repeated freezing and thawing. When stored as directed, product is stable for at least 6 months.

Note: the toxic and carcinogenic ingredients such as solid cacodylate trihydrate and cobalt chloride are contained in the TUNEL Equilibration Buffer and TUNEL Reaction Buffer. Please wear masks and gloves when using.If contacting the skin, please wash with plenty of water immediately. Please treat the waste liquid as toxic substance.

Description

A remarkable feature of apoptosis is the degradation of chromosome DNA, which is a common phenomenon. The degradation is very specific and regular. DNA fragments break into different lengths are of 180 bp-200 bp, showing a specific ladder like ladder pattern in agarose gel electrophoresis. The kit uses TUNEL method and uses Terminal Deoxynucleotidyl Transferase(TdT) catalyzes the incorporation of YF®594-dUTP into the 3′-OH terminal of the broken DNA of apoptotic cells. DNA labeled with YF®594-dUTP can be observed directly by fluorescence microscope or quantified by flow cytometry. TUNEL can selectively detect apoptotic cells rather than necrotic cells or cells with DNA strand breaks caused by irradiation and drug therapy. In this experiment, TdT enzyme catalyzes the incorporation of dUTP into the 3′-OH end of the broken DNA strand. Antigen labeled dUTP (such as digoxin-dUTP, biotin-dUTP) is a more rapid and direct detection method because it can be directly detected in situ.

Protocol

Experimental materials (self provided)

PBS, pH=7.4

4% paraformaldehyde (in PBS)

Bovine serum albumin (BSA) or normal sheep and bovine serum

Solvent for dewaxing (for paraffin section sample)

1. Sample preparation

1.1 Cell sample

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1). Optional: prepare a negative control sample (add TUNEL reaction solution without TdT enzyme).

2). Wash cells twice with PBS.

3). Cell fixation: add appropriate 4% paraformaldehyde, and place at 4 ℃ for 30 min.

4). Wash cells twice with PBS.

5). Cell permeabilization: cells can be permeated with 0.2% Triton X-100 in PBS and placed at room temperature for 20 min.

6). Wash cells twice with PBS.

1.2 Paraffin section

1). Paraffin sections were immersed in dimethylbenzene twice at room temperature for 5 min each time to remove paraffin thoroughly.

Note: dimethylbenzene is toxic and volatile. Please operate in the fume hood.

2). At room temperature, the slices were immersed in absolute ethanol and rinsed twice for 5 min each time.

3). At room temperature, the section samples were immersed in ethanol (95%, 90%, 80%, 70%) of different concentration gradients, and each concentration was rinsed once for 5 minutes.

4). Immerse the slice in pure water for 3 minutes at room temperature, and then immerse the slice in 1 × PBS for 3 minutes. Carefully suck the excess liquid around the slice sample with filter paper.

5). The contour of the sample was drawn around the section sample with an immunohistochemical pen so that it could be penetrated and labeled downstream.

6). Dilute 2 mg/mL of proteinase K solution with 1 × PBS in a ratio of 1:100, so that its final concentration is 20 μg/mL. Add 100 μL diluted proteinase K solution to each sample, make the solution cover all sample areas, and incubate at room temperature for 20 min. The incubation time and temperature of proteinase K need to be optimized according to different types of tissue samples.

Note: protease K can help to penetrate tissues, but prolonging incubation time may cause slices to fall off and too short may cause penetration incomplete, so it is necessary to optimize incubation time. Generally, the time is 10-30 min. Section of 4 μm may need 10 min, section of 30 μm should be extend to 30 min.

7). Infiltrates and cleans the slices twice with PBS for 5 min each time. The excess liquid is sucked by the filter paper, and place processed sample in wet box to keep wet.

Note: in this step, protease K must be washed clean thoroughly, otherwise it will seriously interfere with the subsequent labeling reaction.

1.3 Frozen tissue section

1). Place frozen slices on a shelf at room temperature and dried for 20 minutes.

2). The slides were immersed in 4% polyformaldehyde (in PBS) and fixed at room temperature for 30 min.

3). PBS infiltrates and cleans slices with PBS twice for 5 min each time.

4). Carefully suck the liquid around the sample on the slide with filter paper.

5). Dilute 2 mg/mL of proteinase K solution with 1 × PBS in a ratio of 1:100, so that its final concentration is 20 μg/mL. Add 100 μL diluted proteinase K solution to each sample, make the solution cover all sample areas, and incubate at room temperature for 20 min. The incubation time and temperature of proteinase K need to be optimized according to different types of tissue samples.

Note: protease K can help to penetrate tissues, but prolonging incubation time may cause slices to fall off and too short may cause penetration incomplete, so it is necessary to optimize incubation time. Generally, the time is 10-30 min. Section of 4 μm may need 10 min, section of 30 μm should be extend to 30 min.

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6). Infiltrates and cleans the slices twice with PBS for 5 min each time. The excess liquid is sucked by the filter paper, and place processed sample in wet box to keep wet.

1.4 positive treatment (this step is only for positive control, and other samples are subject to TUNEL reaction directly)

1). Dilute 10 × DNase I buffer with ddH_2O in the proportion of 1:10 to 1 × DNase I buffer for standby.

2). Add 100 μL 1 × DNase I buffer to the transparent sample, and incubate at room temperature for 5 min.

3). Dilute DNase I (2 U/μL) with 1 × DNase I buffer in the proportion of 1:100 to a final concentration of 20 U/mL.

4). Gently suck off the excess liquid, add 100 μL DNase I working solution(20 U/mL) , and incubate at room temperature for 10 minutes.

5). Gently suck off the excess liquid and clean the sample twice with PBS.

2. TUNEL Reaction

2.1 Add 100 μL TUNEL Equilibration Buffer to each sample and incubate for 5 min.

2.2 Prepare TUNEL reaction mixture in advance: Each sample needs 50 μL YF®594 TUNEL Reaction Buffer added with 1 μL TdT enzyme.

2.3 Discard the Equilibration Buffer, carefully suck the excess liquid around the slice sample with filter paper, and add 50μL TUNEL reaction mixture to each sample.

1). Adherent cells: cover the sample evenly with a cover glass. Incubation at 37 ℃ in dark for 60 min.

2). Suspended cells: they can be added to the microplate, incubated with a microplate oscillator or vibrated with a mild reaction tube every 15 minutes to make it react fully. Incubation at 37 ℃ in dark for 60 min.

3). Tissue sample: cover the sample evenly with a cover glass. Place the sample flat in wet box, incubate at 37 ℃ in dark for 2 hours, and lay a paper towel with a small amount of water at the bottom of the wet box to keep humidity.

2.4 Remove the reaction mixture and wash twice with 1 × PBS , each time for 5 minutes. Then wash the sample 3 times with 0.1% Triton X-100 contains 5 mg/mL BSA, each time for 5 min, to reduce the background.

2.5 (Optional) Re-staining: add 2 μg/mL DAPI dye solution to each sample, and incubate at room temperature in dark for 10 min. After dyeing, remove the dye solution gently and soak the sample in 1 × PBS for 3 times, each time for 5 minutes.

2.6 (Optional) Sealing: immerse the slice sample in pure water for 5 min, 70% ethanol for 5 min, 80% ethanol for 5 min, 90% ethanol for 5 min, 95% ethanol for 5 min, and anhydrous ethanol for 5 min，successively. Finally, place the slice sample in the dye cylinder and soak it in fresh xylene for 2 times, each time for 5 min. (operation in ventilation kitchen). After dehydration, wipe off the liquid around the slice, add 50 μL anti fluorescence quenching sealing solution to each slice sample, cover the cover glass, tap the cover glass with the blunt end of the tweezers to remove the bubbles so as to complete the sealing.

2.7 The spectrum of YF555 was similar to that of Texas Red dye. The excitation wavelength and emission wavelength were 593 nm and 614 nm, respectively. (apoptotic cells should be labeled with bright green fluorescence, and negative control samples without TdT enzyme were not labeled with fluorescence).

Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. For your safety and health, please wear lab coats and disposable gloves.

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