Product Information

YF®488/555/594/640/Cy3 TUNEL Apoptosis Kit

Catalog Number and Product Size:

Catalog Number	Product name	Ex/Em (nm)	Size
T6013S	YF®488 TUNEL Apoptosis Kit (Green fluorescence)	490/515	20T
T6013L			50T
T6039S	YF®555 TUNEL Apoptosis Kit (Orange red fluorescence)	555/565	20T
T6039L			50T
T6014S	YF®594 TUNEL Apoptosis Kit (Red fluorescence)	590/617	20T
T6014L			50T
T6063S	YF®640 TUNEL Apoptosis Kit (Far red fluorescence)	642/662	20T
T6063L			50T
T6067S	Cy3 TUNEL Apoptosis Kit	555/565	20T
T6067L			50T

Contents:

Component \ Size	20T	50T
A. TUNEL Equilibration Buffer	2×1 mL	5 mL
B. YF®488/555/594/640/Cy3 TUNEL Reaction Buffer	1 mL	2×1.25 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

Store at -20°C. Component B should be protected from light to avoid repeated freezing and thawing. Expiration date marked on the outer packing.

Description

When cells undergo apoptosis, some DNA endonuclease will be activated, and these endonuclease will cut the genomic DNA between nucleosomes and generate 180 bp-200 bp DNA fragment, which is represented by the specific Ladder pattern presented in agarose gel electrophoresis. Genomic DNA double-strand or single-strand breaks will produce a large number of sticky 3'-OH ends, which can be combined with YF®/Cy-dUTP under the catalysis of deoxyribonucleotide terminal transferase (TdT), thereby passing through Fluorescence microscopy or flow cytometry can directly detect apoptotic cells. This method is called Terminal-

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deoxynucleotidyl transferase mediated nick end labeling (TUNEL). Since normal or proliferating cells have little DNA breakage and thus no 3'-OH formation, they are rarely stained. The Tunel method can perform in situ staining of intact single apoptotic cell nuclei or apoptotic bodies, which can accurately reflect the typical biochemical and morphological characteristics of apoptosis, and can detect a very small amount of apoptotic cells. widely used in research.

This kit has a wide range of applications and can be used to detect apoptosis in frozen or paraffin sections, as well as in cultured adherent cells or suspension cells. Selectively detects apoptotic cells rather than necrotic cells or cells with DNA strand breaks caused by irradiation and drug treatment. This kit detects cell apoptosis, which takes a short time. It only needs one step of staining reaction, and it can be detected after washing.

Protocol

Experimental materials (self provided)

- PBS buffer (1×, pH~7.4)
- 0.2% Triton X-100 (in PBS)
- 0.1% Triton X-100 (in PBS with 5 mg/mL BSA)
- 4% paraformaldehyde (in PBS)
- Pap pen
- Dewaxing solvent (paraffin section samples)
- Reagents for Paraffin Section Processing
- Antifade Mounting Medium
- ddH_2O

Experimental design

A. Positive control (optional):

Positive control slides were prepared by DNase I treatment. DNase I can digest single-stranded or double-stranded DNA to produce single-stranded or double-stranded oligodeoxynucleotide endonucleases, artificially causing apoptosis.

B. Negative control (optional):

Use TUNEL Reaction Buffer without TdT Enzyme and replace TdT Enzyme with ddH_2O.

C. Experimental treatment group.

D. Experimental control group.

Experimental procedure

1. Sample preparation:

(1) For adherent cells or cell smears

a. Wash once with PBS.

Note: If you are concerned that the cells on the cell smear will not stick tightly, you can dry the sample to make the cells stick more tightly.

b. Fixed : add an appropriate amount of 4% paraformaldehyde (in PBS) and fix for 30 minutes at room temperature. Washed twice with PBS.

c. Penetration: add an appropriate amount of 0.2% Triton X-100 (in PBS) and permeabilize for 20 min at room temperature. Washed twice with PBS.

d. Go to step **2. TUNEL reaction**.

(2) For suspension cells or cell suspensions

a. Collect cells (3-5×10^6 cells), centrifuge at 1000 rpm for 5 min, and wash twice with PBS.

b. Fixed : add an appropriate amount of 4% paraformaldehyde (in PBS) to fully resuspend the cells and fix at 4°C for 30 min. Centrifuge at 2000 rpm for 5 min and wash twice with PBS.

c. Penetration: add an appropriate amount of 0.2% Triton X-100 (in PBS) and permeabilize for 20 min at room temperature. Centrifuge at 2000 rpm for 5 min and wash twice with PBS.

d. Go to step **2. TUNEL reaction**.

(3) Paraffin tissue sections

a. Deparaffinization and hydration: put the sliced samples into xylene I (10 min) ➞ xylene II (10 min) ➞ 100% ethanol I (5 min) ➞ 100% ethanol II (5 min) ➞ 95% ethanol (5 min) ➞ 90% ethanol (5 min) ➞ 80% ethanol (5 min) ➞ 70% ethanol (5 min) ➞ ddH_2O for 5 min, rinse twice.

Note: Xylene is toxic and volatile, do this in a fume hood.

b. Use filter paper to blot up the liquid around the sliced sample, and use an Pap pen to circle the outline of the sample for downstream permeabilization and marking.

Note: If it is found that the circle of the immunohistochemical stroke is damaged in the subsequent experimental operation, it needs to be repainted in time.

c. Penetration: dilute 2 mg/mL Proteinase K solution with PBS to a final concentration of 20 μg/mL at a ratio of 1:100, add 100 μL dropwise to each sample so that the solution covers the entire sample area, 20-37°C Incubate for 20 min.

Note: Proteinase K permeates the cell and nuclear membranes, thus allowing the staining reagents in the subsequent steps to enter the nucleus for reaction and improving labeling efficiency. Too long incubation time will increase the risk of tissue sections falling off the slide during subsequent washing steps, while too short incubation time may result in insufficient permeabilization and affect labeling efficiency. For better results, the concentration of Proteinase K, incubation time, and temperature should be optimized for different types of tissue samples.

d. Rinse the sections twice with PBS for 5 min each time, absorb the excess liquid with filter paper, and place the processed samples in a wet box to keep them moist.

Note: Proteinase K must be washed in this step, otherwise it will seriously interfere with subsequent labeling reactions.

e. Go to step **2. TUNEL reaction**.

(4) Frozen tissue sections

a. Fixed: remove frozen sections and allow to warm to room temperature. An appropriate amount of 4% paraformaldehyde (in PBS) was added and fixed at room temperature for 30 min. Rinse twice with PBS for 10 min each.

Note: If you are worried that the formaldehyde is not cleaned properly, it will affect the final dyeing effect. After the fixation with formaldehyde, an appropriate amount of 2 mg/mL glycine can be added for 10 min to neutralize the residual fixative, and then washed with PBS.

b. Use filter paper to blot up the liquid around the sliced sample, and use an Pap pen to circle the outline of the sample for downstream permeabilization and marking.

Note: If it is found that the circle of the immunohistochemical stroke is damaged in the subsequent experimental operation, it needs to be repainted in time.

c. Penetration: dilute 2 mg/mL Proteinase K solution with PBS to a final concentration of 20 μg/mL at a ratio of 1:100, add 100 μL dropwise to each sample so that the solution covers the entire sample area, 20-37°C Incubate for 20 min.

Note: Proteinase K permeates the cell and nuclear membranes, thus allowing the staining reagents in the subsequent steps to enter the nucleus for reaction and improving labeling efficiency. Too long incubation time will increase the risk of tissue sections falling off the slide during subsequent washing steps, while too short incubation time may result in insufficient permeabilization and affect labeling efficiency. For better results, the concentration of Proteinase K, incubation time, and temperature should be optimized for different types of tissue samples.

d. Rinse the sections twice with PBS for 5 min each time, absorb the excess liquid with filter paper, and place the processed samples in a wet box to keep them moist.

Note: Proteinase K must be washed in this step, otherwise it will seriously interfere with subsequent labeling reactions.

e. Go to step **2. TUNEL reaction**.

(5) Positive treatment (only the positive control is subjected to this step, and other samples are directly subjected to the TUNEL reaction step)

a. Dilute 10× DNase I Buffer with ddH_2O at a ratio of 1:10 to 1× DNase I Buffer for later use.

b. Add 100 μL of 1× DNase I Buffer dropwise to the treated sample, covering the entire sample area, and equilibrate for 5 min at room temperature.

c. Dilute DNase I (2 U/μL) 1:100 with 1× DNase I Buffer to a working solution with a final concentration of 20 U/mL.

d. Discard Buffer, add 100 μL of DNase I working solution at a concentration of 20 U/mL, and incubate for 10 min at room temperature.

e. Discard DNase I working solution and wash 2 times with PBS.

f. Go to step **2. TUNEL reaction**.

2. TUNEL Reaction

(1) Add 100 μL TUNEL Equilibration Buffer to each sample and incubate for 5 min.

(2) Prepare TUNEL reaction solution (ready to use):

	1 sample	5 samples	10 samples
TdT Enzyme	1 μL	5 μL	10 μL
YF®488/555/594/640/Cy3 TUNEL Reaction Buffer	49 μL	245 μL	490 μL
Total volume of TUNEL reaction solution	50 μL	250 μL	500 μL

(3) For adherent cells, cell smears or tissue sections

a. Discard the TUNEL Equilibration Buffer and add 50 μL of TUNEL reaction solution to each sample to cover the sample uniformly.

Incubate for 60 min at 37°C, protected from light.

Note: 50 μL of TUNEL reaction solution is suitable for smears, slices or 96-well plates (the volume of TUNEL reaction solution can be adjusted appropriately for other orifice plates, just cover cells). If the sample to be tested is a smear, slice, or in a 24-well, 12- or 6-well plate, you can use an anti-evaporation film, or try to use a ziplock bag or other suitable material and cut it into a circle slightly smaller than the hole. It is a plastic sheet, and the TUNEL reaction solution is dripped and covered on the sample, which can prevent the evaporation of the TUNEL reaction solution and make the TUNEL reaction solution evenly cover the sample.

b. Discard the TUNEL reaction solution and rinse with PBS twice for 5 min each.

Note: In order to reduce the background, after rinsing the sections once with PBS, you can rinse the sections with 0.1% Triton X-100 (in PBS with 5 mg/mL BSA) for 3 times for 5 min each time, in this way, free unreacted label can be removed cleanly.

c. (Optional) Add an appropriate amount of DAPI staining solution with a concentration of 5 μg/mL to each sample, and incubate at room temperature for 5 min in the dark. After staining, the DAPI staining solution was discarded, and PBS was rinsed twice for 5 min each.

d. (Optional) Section sealing: Immerse the sections in pure water, 70% ethanol, 80% ethanol, 90% ethanol, 95% ethanol, and absolute ethanol for 5 minutes, and finally place the sectioned samples in a staining jar Soak in fresh xylene, and clear for 2 times, 5 min each time. After dehydration is complete, wipe off the liquid around the sections, drop 50 μL of Antifade Mounting Medium into each sample (Antifade Mounting Medium may not be suitable for some dyes, it is recommended to conduct pre-experimental test matching before experiment), cover with a coverslip, and tap the coverslip with the blunt end of tweezers to remove air bubbles to seal. slice completely.

e. Absorb excess liquid with filter paper, add 100 μL of PBS to the sample area to keep the sample moist, and observe immediately under a fluorescence microscope.

(4) For suspension cells or cell suspensions

a. Centrifuge at 2000 rpm for 5 min, discard the TUNEL Equilibration Buffer, add 50 μL of TUNEL reaction solution to each sample tube to gently resuspend the cells, and incubate at 37°C for 60 min in the dark. Gently resuspend cells with a micropipette every 15 min.

b. Centrifuge at 2000 rpm for 5 min, discard the TUNEL reaction solution, add an appropriate amount of 0.1% Triton X-100 (prepared in PBS, containing 5 mg/mL BSA) to gently resuspend the cells, and wash twice.

c. Add 100 μL of DAPI staining solution at a concentration of 5 μg/mL to each sample tube, and incubate at room temperature for 5 min in the dark.

d. Add 400 μL of PBS to resuspend the cells, and immediately detect by flow cytometry or observe under a fluorescence microscope after smearing.

Notes

1. Before use, please centrifuge the product to the bottom of the tube briefly, and then perform subsequent experiments.

2. When using components A and B, please wear masks and gloves, and if they touch the skin, please rinse immediately with plenty of water.

3. Fluorescent dyes all have quenching problems, please avoid light as much as possible to slow down fluorescence quenching.

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4. For your safety and health, please wear a lab coat and disposable gloves.

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