Aladdin®488 TUNEL apoptosis Kit (green fluorescence)

A598363

Storage Store at-20°C. Component A must be kept away from light and avoid repeated freeze-thaw cycles. See outer packaging for expiration date. Transport with ice packs.

Applied range

Apoptosis detection.

Product presentation

During apoptosis, certain DNA endonucleases are activated, which cleave genomics DNA between nucleoside, producing DNA fragments of 180 bp to 200 bp. These fragments exhibit a characteristic ladder pattern on agarose gel electrophoresis. When genomic DNA undergoes double-strand or single-strand breaks, a large number of sticky 3'-OH ends are generated. These can be bound to YF®-dUTP under the catalytic action of deoxynucleotidyl transferase (TdT), enabling direct detection of apoptotic cells via fluorescence microscopy or flow cytometry. This method is termed terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL).Since normal or proliferating cells have almost no DNA breaks, 3'-OH formation is absent, and thus they are rarely stained.The Tunel method enables in situ staining of intact individual apoptotic nuclei or apoptotic bodies, accurately reflecting the typical biochemical and morphological characteristics of apoptosis. It can detect even the minimal number of apoptotic cells, making it widely adopted in apoptosis research.This kit has a wide range of applications and can be used to detect apoptosis in frozen or paraffin-embedded sections, as well as in cultured adherent or suspension cells.Selective detection of apoptotic cells, rather than necrotic cells or cells with DNA strand breaks caused by irradiation and drug therapy.This kit detects apoptosis with a short reaction time, requiring only one staining step followed by washing for immediate detection.

Usage method

Experimental materials (self-provided)

PBS buffer solution

（1×，pH~7.4）

0.4%Triton X-100（PBS compound）

0.1%Triton X-100（PBS compound，include 5 mg/ml BSA）

4% paraformaldehyde（PBS compound）

Immunohistochemical pen
Wax removal solvent (paraffin section sample)
Reagents for paraffin section processing
anti-fluorescence quenching stopper
ddH2O

experimental design

A. positive control

The positive control slides were prepared by DNase I treatment.DNase I can digest single-stranded or double-stranded DNA, exposing the 3'-OH ends and artificially inducing apoptosis.Perform one run per experiment.(To verify whether there are any issues with the experimental procedures and reagent kit)

B. negative control

Use TUNEL Reaction Buffer without TdT enzyme, replacing TdT enzyme with ddH2O.(Primarily to exclude nonspecific staining caused by cellular apoptosis, procedural factors, etc.; and to adjust the exposure intensity of the imaging.)

C. Experimental treatment group

The experimental group was operated according to the instructions.

D. Experimental control group

The experimental group followed the instructions for normal operation.

experimental procedure

1. Sample preparation

(1)For adherent cells or cell smears

a. Wash once with PBS

Note: If concerned about poor cell adhesion in the smear, drying the sample may enhance cell attachment.

b. Fixation: Add an appropriate amount of 4% polyformaldehyde (PBS solution) and fix at 4°C for 30 minutes.PBS was used for two washes.

c.Permeabilization: Add an appropriate amount of 0.4% Triton X-100 (PBS solution) and permeabilize at room temperature for 20 minutes.The samples were washed twice with PBS.

d. Go to Step 2.TUNEL reaction.

(2)for suspended cells or cell suspensions

a. collecting cell

(3-5×10 6 cells),1000 rpm Centrifuge 5 min,Wash twice with PBS

b. Fixation: Add an appropriate amount of 4% polyformaldehyde (PBS-prepared) to fully resuspend the cells, and fix at 4°C for 30 minutes.Centrifugation at 2000 rpm for 5 minutes, followed by two washes with PBS.

c. Permeabilization: Add an appropriate amount of 0.4% Triton X-100 (PBS solution) and permeabilize at room temperature for 20 minutes.Centrifugation at 2000 rpm for 5 minutes, followed by two washes with PBS.
d. Go to Step 2.TUNEL reaction.
(3)paraffin section
a. The paraffin sections were placed on a slide holder and dried in a 60℃ oven for 60 minutes.
b. Wax removal and hydration: The sliced samples were sequentially placed in xylene I(10 min)→dimethylbenzeneII(10 min)→100%alcoholI(5 min)→100%alcoholII(5 min)→ 95%alcohol (5 min)→ 90%alcohol (5 min)→ 80%alcohol (5 min)→ 70%alcohol (5 min)→ ddH2O wash 5 min,wash 2 times
Note: Xylene is toxic and highly volatile. Perform this operation in a fume hood.
c. Absorb the surrounding liquid of the section sample with filter paper, and outline the sample contour with an immunohistochemical pen for downstream permeability and labeling.
Note: If the contour circle of the immunohistochemical brush stroke is found to be damaged during subsequent experimental procedures, it should be promptly redrawn.
d. Permeability: Dilute the 2 mg/mL Proteinase K solution with PBS at a ratio of 1:50 to achieve a final concentration of 40 μg/mL. Add 100 μL to each sample to cover the entire sample area, and incubate at 37°C for 30 minutes.
Note: Proteinase K permeates both the cell membrane and nuclear membrane, enabling subsequent staining reagents to fully enter the nucleus for reaction, thereby enhancing labeling efficiency.Prolonged incubation time increases the risk of tissue sections detaching from the carrier slide during subsequent washing steps, while insufficient incubation may result in inadequate permeabilization, thereby compromising labeling efficiency.To achieve better results, the concentration of Proteinase K, incubation time, and temperature should be optimized according to different types of tissue samples.
e.Immerse the sliced samples in 1×PBS for three rinses, each for 5 minutes, then absorb excess liquid with filter paper. Place the processed samples in a moist box to maintain humidity.
Note: Proteinase K must be thoroughly washed off at this step, as it could significantly interfere with subsequent labeling reactions.
f. Go to Step 2.TUNEL reaction.
(4)Frozen tissue section
a. Fixation: Remove the frozen sections and return to room temperature.Immerse the sectioned samples in 4% polyformaldehyde (PBS-buffered) and fix at room temperature for 30 minutes.The tissue sections were rinsed three times with 1×PBS, each time for 10 minutes.

Note: If concerned about incomplete formaldehyde cleaning, which may affect the final dyeing effect.After formaldehyde fixation, add an appropriate amount of 2 mg/mL glycine to wash for 10 minutes to neutralize residual fixative, followed by PBS washing.
b. Absorb the surrounding liquid of the section sample with filter paper, and outline the sample contour with an immunohistochemical pen for downstream permeability and labeling.
Note: If the contour circle of the immunohistochemical brush stroke is found to be damaged during subsequent experimental operations, it should be promptly redrawn.
c. Permeability: Dilute the 2 mg/mL Proteinase K solution with PBS at a ratio of 1:50 to achieve a final concentration of 40 μg/mL. Add 100 μL to each sample to cover the entire sample area, and incubate at 37°C for 20 minutes.
Note: Proteinase K permeates both the cell membrane and nuclear membrane, enabling subsequent staining reagents to fully enter the nucleus for reaction, thereby enhancing labeling efficiency.Prolonged incubation time increases the risk of tissue sections detaching from the carrier slide during subsequent washing steps, while insufficient incubation may result in inadequate permeabilization, thereby compromising labeling efficiency.To achieve better results, the concentration of Proteinase K and incubation time,
The temperature should be optimized according to different types of tissue samples.If optimizing the concentration of Proteinase K and incubation time still fails to improve staining results, the sample can be immersed in 1% Triton X-100 (PBS-buffered) for 3-5 minutes at room temperature to promote permeation. Subsequently, the section samples should be rinsed three times with 1×PBS, each time for 5 minutes.
d. Immerse the sliced samples in 1×PBS for three rinses, each for 5 minutes, then absorb excess liquid with filter paper. Place the processed samples in a moist box to maintain humidity.
Note: Proteinase K must be thoroughly washed off at this step, as it could significantly interfere with subsequent labeling reactions.
e. Go to Step 2.TUNEL reaction
(5)Positive treatment (only the positive control undergoes this step, while other samples proceed directly to the TUNEL reaction step)
a. Dilute 10×DNase I Buffer with ddH2O at a ratio of 1:10 to prepare 1×DNase I Buffer.
b. Add 100 μL of 1×DNase I Buffer to the treated samples, covering the entire sample area, and incubate at room temperature for 5 minutes.
c. Dilute DNase I (2 U/μL) with 1×DNase I Buffer at a ratio of 1:100 to achieve a working solution with a final concentration of 20 U/mL.
d. Remove the buffer, add 100 μL of DNase I working solution (concentration: 20 U/mL), and incubate at room temperature for 15 minutes.
e. Remove the DNase I working solution and wash twice with PBS.
f. Go to Step 2.TUNEL reaction.

2. TUNEL reaction

(1)Prepare TUNEL reaction mixtures (ready-to-use)

A598363	1 sample	5 sample	10 sample
TdT enzyme	2 μL	10 μL	20 μL
ALADDIN® 488 TUNEL Reaction Buffer	48 μL	240 μL	480 μL
Total volume of TUNEL reaction mixture	50 μL	250 μL	500 μL

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

a. Add 50 μL of TUNEL reaction buffer to each sample to ensure uniform coverage.Incubate at 37°C in a light-protected environment for the recommended duration (15-30 minutes for cell staining, 1 hour for tissue staining).

Note: 50 μL of TUNEL reaction mixture is suitable for smears, sections, or 96-well plates (the reaction volume can be adjusted for other plate types as needed to cover the cells).For samples to be tested, such as smears, sections, or those in 24-well, 12-well, or 6-well plates, an anti-evaporation film can be used, or alternatively, self-sealing bags or other suitable materials can be cut into slightly smaller circular plastic sheets than the wells. After adding the TUNEL reaction solution, these sheets can be placed over the samples to prevent evaporation of the TUNEL reaction solution and ensure uniform coverage of the sample.

b. After discarding the TUNEL reaction mixture, the samples were washed twice with PBS, followed by three washes with 0.1% Triton X-100 (PBS-buffered solution containing 5 mg/mL BSA) for 5 minutes each to effectively remove unbound labeled molecules.

c.(Optional) Add an appropriate volume of 5 μg/mL DAPI staining solution to each sample and incubate at room temperature in the dark for 5 minutes.After staining, discard the DAPI solution and wash with PBS twice, each time for 5 minutes.

d.(Optional) Slide sealing: Add 20 μL of anti-fluorescence quenching sealing reagent per sample (Note: The anti-fluorescence quenching sealing reagent may not be compatible with certain dyes; pre-experiment testing is recommended to confirm compatibility). Cover the slide with a cover glass and gently tap the cover glass with the blunt end of forceps to remove air bubbles, ensuring complete sealing.

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

(3)for suspended cells or cell suspensions

a.Add 50 μL of TUNEL reaction buffer to each sample tube to gently resuspend the cells, then incubate at 37°C in a dark place for 15-30 minutes.The cells were gently resuspended with a micropipette every 10 minutes.

b.Centrifuge at 2000 rpm for 5 minutes, discard the TUNEL reaction mixture, and wash twice with 0.1% Triton X-100 (PBS containing 5 mg/mL BSA) for 5 minutes each time to effectively remove unbound unlabeled molecules.
c. Add 100 μL of 5 μg/mL DAPI dye to each sample tube and incubate at room temperature in the dark for 5 minutes.
d. Add 400 μL PBS to resuspend the cells, and immediately perform flow cytometry detection or prepare a smear for observation under a fluorescence microscope.

Matters need attention

1. Prior to use, centrifuge the product to the bottom of the tube for a brief period before proceeding with subsequent experiments.
2. The staining time can be reduced when the background staining is heavy or the non-specific staining is obvious.
3. Negative control and positive control groups should be added in the experiment.
4. When using Component A, wear a mask and gloves. If it comes into contact with the skin, rinse immediately with plenty of water.
5. All fluorescent dyes exhibit quenching issues. Please ensure light protection to mitigate fluorescence quenching.
6. This product is intended for scientific research only and shall not be used for clinical diagnosis or treatment, nor shall it be used in food or pharmaceutical products, or stored in ordinary residential premises.
7. For your safety and health, wear lab coats and disposable gloves when handling.

FAQ

1. Why do some nonspecific markers appear?

1)Certain cells, such as those with high levels of smooth muscle nuclease or polymerase activity, may result in complete DNA cleavage, which can easily lead to false positives.Recommendation: Immediately fix and fully immobilize the cells after extraction to inhibit the activity of these enzymes, while setting up a negative control.
2)The fixation medium concentration is either too high or too low, resulting in poor fixation of cells in the central region, leading to autolysis of central cells and irregular DNA strand breaks, which produce false-positive results.Recommendation: 4% polyformaldehyde is recommended.
3)Prolonged TdT reaction time or evaporation/leakage of reaction mixture during the Tunel assay prevents adequate cell surface hydration.Pay attention to reaction time and ensure the TdT enzyme reaction solution adequately covers the sample.
4)Some reagents or cells exhibit autofluorescence, and the selection of dyes should avoid the color of autofluorescence.

2. Why were the cells not stained with fluorescent dyes?

1)Not properly fixed.The preferred fixative is 4% polyformaldehyde, which should be prepared immediately before use.The use of ethanol is not recommended, as ethanol fixative has weak permeability, which affects the efficiency of TUNEL labeling.
2)The membrane permeability of the cell membrane and nuclear membrane is insufficient, and TdT enzyme cannot reach the nucleus.Cells can be permeabilized with 0.2% Triton X-100 solution for 5-30 minutes, with the permeabilization time varying slightly depending on the cell type and requiring appropriate adjustment.
3)The extension time was extended to 2 hours, and the amounts of TdT and dUTP were appropriately increased.
4)The apoptosis of the experimental cells was confirmed.Prepare a DNase I-treated positive control to verify the proper execution of the TdT reaction.
3. How to perform double staining of the nucleus?The nuclei can be stained after the TUNEL reaction is completed.
4. Why is the fluorescence background high?
1)Mycoplasma contamination: The Mycoplasma staining detection kit can be used for verification.
2)If the TdT reaction time is prolonged, dilute the TdT enzyme 2-5 times with the dilution solution provided in the kit, then proceed according to the instructions. The diluted TdT enzyme should only be used on the same day.
3)DNA breaks in the nucleus can also occur in cells that are in a state of rapid division and proliferation.Recommendation: Sampling should be conducted during non-high proliferation periods;
4)Some reagents or cells exhibit autofluorescence, and the selection of dyes should avoid the color of autofluorescence.
5)Prolonged DAB incubation reduces DAB staining time.
6)After the TdT reaction, the non-specific binding of biotin-X-dUTP was washed three times with PBS containing 0.1% Triton X-100 and 1 mg/ml BSA.
5. Low detection rate?
1)Samples fixed with ethanol, methanol, or formaldehyde (most commercially available formaldehyde solutions contain methanol) exhibit lower labeling efficiency (due to the failure of chromatin to cross-link with proteins during fixation, resulting in loss during handling). The recommended fixative should be used.
2)The fixation time is too long, resulting in excessive cross-linking. Reduce the fixation time.
3)If drugs are used to induce apoptosis in adherent cells, the cells will shrink, and the adhesion force will decrease, making the apoptotic cells prone to detachment.Recommendation: After apoptosis induction is completed, the microplate can be centrifuged at 1000g for 5 minutes, followed by medium aspiration and PBS washing.If no suitable centrifuge is available, handle gently to prevent apoptotic cells from being washed away during the wash cycle.The whole operation should be gentle.