Biotin TUNEL Apoptosis Kit

Catalog No.: RA20086

Basic Information

Product name	Biotin TUNEL Apoptosis Kit
Sizes	20T/50T
Storage	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

During apoptosis, certain DNA endonucleases are activated to cleave genomic DNA between nucleosomes, generating 180 bp-200 bp fragments. These fragmented DNA strands expose numerous mucous 3'-OH termini. Under the catalytic action of deoxyribonucleotide terminal transferase (TdT), these fragments bind to biotin-dUTP, then combine with streptavidin-HRP-labeled horseradish peroxidase (HRP). The HRP-catalyzed DAB coloration ultimately reveals apoptotic cells through optical microscopy. This method is termed Terminal-deoxynucleotidyl Transferase-mediated dUTP nick end labeling (TUNEL) for direct detection of apoptotic cells.

This kit demonstrates broad applicability, enabling analysis of apoptosis in both frozen/wax sections and cultured adherent or suspension cells. It selectively detects apoptotic cells rather than necrotic cells or those with DNA strand breaks caused by radiation or drug treatment.

Product Components

components	20 T	50 T
A. Biotin TUNEL Reaction Buffer	1 mL	2 × 1.25 mL
B. TdT Enzyme	40 μL	100 μL
C. Streptavidin-HRP	20 μL	50 μL
D. Streptavidin-HRPdiluent	1 mL	2 × 1.25 mL
E. DAB concentrate（20×）	50 μL	125 μL
F. DAB diluent	1 mL	2 × 1.25 mL
G.Color enhancer（10×）	100 μL	250 μL
H. Proteinase K（2 mg/mL）	40 μL	100 μL
I. DNase I（2 U/μL）	5 μL	13 μL
J. 10× DNase I Buffer	100 μL	260 μL

Biotin TUNEL Apoptosis Kit

Catalog No.: RA20086

Note:

(1)Components A, C, E, and F must be stored in a dark place.

(2)Components B, C, H, and I require ice-cold handling.

(3)Component F should avoid repeated freezing-thaw cycles and is recommended for separate storage.

(4)Before use, centrifuge the product briefly until it settles at the bottom of the tube before proceeding with subsequent experiments.

Materials Required (Not Supplied)

1.PBS buffer (1×, pH 7.4)

2. 1% Triton X-100 (diluted in PBS)

3. Paraffin section processing reagents

4.4% polyformaldehyde (diluted in PBS)

5.Immunohistochemistry pen

6.3% H2O2 (methanol:ddH2O:H2O2 = 8:1:1; freshly prepared)

7. Neutral resin

design of experiments

A.Positive Control (optional): Prepare positive control slides using DNase I treatment. DNase I is a nucleases that digest single-stranded or double-stranded DNA to producsingle-deoxyribonucleotides, or generate single-stranded or double-stranded oligodeoxyribonucleotides, thereby inducing artificial apoptosis.

B. Negative Control (optional): Use Biotin TUNEL Reaction Buffer without TdT Enzyme, substituting TdT Enzyme with ddH2O.

C. Experimental Treatment Group.

D. Experimental Control Group.

Perimental procedure

1.Sample Preparation:

(1) For adherent cells or cell slides

a. PBS wash once.

Note: If cell adhesion is compromised, dry the sample to enhance adhesion.

b. Fixation: Add 4% polyformaldehyde (PBS buffer) and fix for 30 min at 4℃. Wash twice with PBS.

c. Permeabilization: Add 0.4% Triton X-100 (PBS buffer) and permeabilize at room temperature for 20 min. Wash twice with PBS.
d. Inactivation: Add approximately 100 μL 3% H2O2 per well, cover completely with cells, and incubate at room temperature in the dark for 20 min to inactivate endogenous catalase. Wash twice with PBS.
e. Proceed to Step 2: TUNEL reaction.
(2) For suspension cells or cell cultures
a. Collect cells (3-5×10^6 cells), centrifuge at 1000 rpm for 5 minutes, and wash twice with PBS.
b. Fixation: Add 4% polyformaldehyde (PBS buffer) and resuspend cells. Fix for 30 min at 4℃. Centrifuge at 2000 rpm for 5 minutes, then wash twice with PBS.
c. Permeabilization: Add 0.4% Triton X-100 (PBS buffer) and permeabilize at room temperature for 20 min. Centrifuge at 2000 rpm for 5 minutes, then wash twice with PBS.
d. Sealing: Add approximately 100 μL of 3% H2O2 solution per well. Gently aspirate to resuspend the cells, then incubate at room temperature in the dark for 20 minutes to inactivate endogenous catalase. Subsequently, wash twice with PBS.
e. Proceed to step 2: TUNEL reaction.
(3) Paraffin Sections
a. Drying: Place the section in an oven at 60℃°C for 1 hour.
b. De-waxing and Waterization: Submerge the section sequentially into 100% ethanol I (5 minutes) → 100% ethanol II (2 minutes) → 95% ethanol (5 minutes) → 90% ethanol (5 minutes) → 80% ethanol (5 minutes) → 70% ethanol (5 minutes) → ddH2O (5 minutes) → PBS (5 minutes).
Note: Xylene is toxic and volatile. Perform this step in a fume hood.
c. Dry the liquid around the section using filter paper and outline the sample with an immunohistochemistry pen for subsequent permeabilization and labeling.
Note: If the pen outlines are damaged during later experiments, retrace them promptly.
d. Permeabilization: Dilute 2 mg/mL Proteinase K solution with PBS or ddH2O to 40 μg/mL using a 1:50 dilution ratio. Add 100 μL per sample to ensure full coverage. Incubate at 37℃°C for 30 minutes.
Note: Proteinase K permeates cell membranes and nuclear membranes, allowing subsequent staining reagents to fully enter nuclei for reactions and improve labeling efficiency. Extended incubation increases the risk of tissue detachment during washing, while insufficient permeabilization may compromise labeling effectiveness. To obtain optimal results, the concentrations of Proteinase K, incubation time, and temperature should be optimized according to different tissue types.
e. PBS washing: Wash sections three times with 5-minute intervals.
Note: This step must thoroughly rinse Proteinase K to prevent interference with subsequent

labeling reactions.

f. Block: Submerge sections in 3% H2O2 solution at room temperature (15-25℃) for 20 minutes to inactivate endogenous catalase.

g. PBS washing: Wash sections three times with 5-minute intervals, blot excess liquid on filter paper, and maintain moistness in humid incubation box. h. Proceed to Step 2: TUNEL reaction.

(4) Frozen tissue sections

a. Fixation: Remove frozen sections and thaw at room temperature (no moisture on slides). Immerse in 4% polyformaldehyde (PBS buffer) at room temperature for 30 minutes. PBS rinse three times slowly (5 minutes each).

Note: To ensure thorough fixation, add 2 mg/mL glycine for 10 minutes after formaldehyde fixation to neutralize residual fixative before PBS rinsing.

b. Permeabilization: Immerse sections in 1% Triton X-100 (PBS buffer) at room temperature for 5 minutes.

Note: Extended incubation increases risk of tissue detachment during subsequent washing steps, while insufficient time may compromise permeabilization efficiency.

c. PBS rinse the sections three times, each for 5 minutes. d. Inactivation: Submerge the section samples in 3% H2O2 solution and incubate at room temperature for 20 minutes to deactivate endogenous catalase.

e. PBS rinse the sections three times, each for 5 minutes. Absorb excess liquid around the sections using filter paper, then outline the sample contours with an immunohistochemistry pen for subsequent labeling. Place the processed sections in a humid chamber to maintain moisture.

Note: If the contour outlines of the immunohistochemistry pen are damaged during later experimental procedures, they should be promptly redone. f. Proceed to step 2. TUNEL reaction.

(5)Positive control treatment (This step is only performed for positive controls; other samples proceed directly to the TUNEL reaction step)

a. Dilute 10× DNase I Buffer with ddH2O at a 1:10 ratio to prepare 1× DNase I Buffer for later use.

b. Add 100 µL 1× DNase I Buffer to the treated samples, cover the entire sample area, and allow equilibrium at room temperature for 5 minutes.

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

d. Discard the buffer and add 100 µL of DNase I working solution at 20 U/mL. Incubate at room temperature or 37°C for 15-30 minutes (For reference only: cell samples can be incubated at room temperature for 15 mim; section samples can be incubated at 37 C for 30min).

e. Discard the DNase I working solution and rinse with PBS three times, each time for 5 minutes.

f. Proceed to step 2: TUNEL reaction.

2.TUNEL reaction

(1) Preparation of TUNEL reaction solution (made and used at the same time):

TUNEL Reagent preparation	1 sample	5 sample	10 sample
TdT enzyme	2 μL	10 μL	20 μL
Biotin TUNEL Reaction Buffer	48 μL	240 μL	480 μL
Total volume	50 μL	250 μL	500 μL

(2)Add 50 μL TUNEL reaction solution to each sample to ensure uniform coverage, then incubate at 37℃°C for 60 minutes.

Note: The 50 μL TUNEL solution is suitable for smears, slides, or 96-well plates (other plate types may adjust the solution volume to cover cells). For samples in smears, slides, 24-well plates, 12-well plates, or 6-well plates, use an evaporation-proof film. Alternatively, you may cut slightly smaller circular plastic sheets than the wells using self-sealing bags or other suitable materials. After adding the TUNEL solution, cover the sample with these sheets to prevent evaporation and ensure uniform coverage.

(3)Discard the TUNEL solution, wash with PBS once, then rinse three times with PBS containing 0.1% TritonX-100 and 5 mg/mL BSA.

Preparation of Streptavidin-HRP Working Solution and DAB Colorant Solution

(1) Preparation of Streptavidin-HRP Working Solution (freshly prepared and used):

Preparation of streptavidin-HRP working fluid	1 sample	5 sample	10 sample
Streptavidin-HRP	1 μL	5 μL	10 μL
Streptavidin-HRP diluent	49 μL	245 μL	490 μL
Total volume	50 μL	250 μL	500 μL

(2) Preparation of DAB colorant (make and use at the same time):

DAB colorant preparation	1 sample	5 sample	10 sample
DAB Concentrate (20×)	2.5 μL	12.5 μL	25 μL
DAB diluent	47.5 μL	237.5 μL	475 μL
Total volume	50 μL	250 μL	500 μL

Note: 1) Avoid using sodium azide-containing buffers, as sodium azide acts as an HRP inhibitor. 2)DAB concentrate (20×) and DAB dilution must be thoroughly dissolved and mixed before use. 3) Prepared DAB chromogenic solution can be stored at 4℃ for up to 5 days under light protection. Any sedimentation in working solution does not affect staining. For solutions showing sedimentation, centrifuge at high speed and use only the supernatant.

(3) Preparation of DAB chromogenic enhancement solution (use immediately):

DAB color enhancement preparation	1 sample	5 sample	10 sample
Color enhancer (10×)	5 μL	25 μL	50 μL
ddH2O	45 μL	225 μL	450 μL
Total volume	50 μL	250 μL	500 μL

Note: The chromogenic enhancer (10×) must be thoroughly mixed before use.

4.Sample Color Development

(1) Add 50 μL Streptavidin-HRP working solution to each sample and incubate at 37℃°C in the dark for 30 minutes.

Note: The 50 μL Streptavidin-HRP working solution is suitable for smears, sections, or 96-well plates (other plate types may adjust the working solution volume to cover cells appropriately). To prevent evaporation, an anti-evaporation film should be placed over the sample.

(2)Discard the Streptavidin-HRP working solution and wash with PBS three times.

(3) Add 50 μL DAB chromogenic reagent to each sample and incubate at room temperature in the dark for 1-30 minutes or longer, depending on color development. If no background appears, continue incubation until desired intensity is achieved.
(4) Remove the DAB staining reagent and rinse the sample 3-5 times with ddH2O/PBS to terminate the color reaction.
(5) (Optional) For faint DAB color or enhanced signals:
a. After final washing, remove ddH2O/PBS and add 50 μL chromogenic enhancer to ensure full sample coverage. 1. Incubate at room temperature in the dark for 1-30 minutes or longer, depending on the sample's color development until the light brown precipitate formed by DAB at the epitope becomes dark brown without significant nonspecific or background staining.
b. Remove the chromogenic enhancer and rinse the sample 3-5 times with ddH2O/PBS to terminate the enhanced color development reaction.
(6) (Optional) Slicing: Add appropriate amounts of hematoxylin or methyl green staining solution for nuclear staining. Discard the staining solution and rinse twice with PBS.
(7) (Optional) Mounting: Drop 50 μL neutral resin onto each sample, cover with a coverslip, and gently tap the coverslip with the blunt end of forceps to remove air bubbles for complete mounting.
(8) Absorb excess liquid with filter paper, add 100 μL PBS to the sample area to keep the sample moist, and analyze the sample immediately under the optical microscope.

Notes

1.Before use, centrifuge the product to the tube bottom immediately before proceeding with subsequent experiments.
2. Sodium azide inhibits HRP activity; therefore, do not use reagents containing sodium azide in experiments.
3. For your safety and health, wear lab coats and disposable gloves during operation.
4. This product is for scientific research purposes only and must not be used for clinical diagnosis or treatment.

This product is for research use only!