One-Step Universal TUNEL Apoptosis Kits(Fluorescein 555, Red-Orange)

Catalog No.: RA20051

Basic Information

Product name	One-Step Universal TUNEL Apoptosis Kits(Fluorescein 555, Red-Orange)
Sizes	20 T/50 T
Storage	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	555/565 nm

Product Introduction

During apoptosis, activated intracellular endonucleases cleave genomic DNA between nucleosomes, producing DNA fragments of 180–200 bp, which appear as a characteristic ladder pattern in agarose gel electrophoresis. When genomic DNA is broken (double- or single-stranded), a large number of sticky 3'-OH termini are generated. These termini can be labeled with dUTP under the catalysis of terminal deoxynucleotidyl transferase (TdT). This allows direct detection of apoptotic cells using fluorescence microscopy or flow cytometry. This method is known as Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL). Since normal or proliferating cells rarely have DNA breaks, 3'-OH termini are almost absent, resulting in minimal background staining. The TUNEL assay enables in situ labeling of intact apoptotic nuclei or apoptotic bodies, accurately reflecting the biochemical and morphological characteristics of apoptosis. It is highly sensitive and capable of detecting even a small number of apoptotic cells, making it widely used in apoptosis research.

This kit has a broad range of applications. It can be used to detect apoptosis in frozen or paraffin-embedded tissue sections, as well as in adherent or suspension cell cultures. It selectively labels apoptotic cells, distinguishing them from necrotic cells or cells with DNA strand breaks caused by irradiation or drug treatment. The assay is rapid, requiring only a single staining step followed by washing and detection.

Product Components

Components	20 T	50 T
A. 555 TUNEL Reaction Buffer	1 mL	2×1.25 mL
B. TdT Enzyme	40 μL	100 μL

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C. Proteinase K (2 mg/mL)	40 μL	100 μL
D. DNase I (2 U/μL)	5 μL	13 μL
E. 10 × DNase I Buffer	100 μL	260 μL

Note: Component A must be protected from light and avoid repeated freeze-thaw cycles.

Materials Required (Not Supplied)

- PBS buffer（1×，pH~7.4）
- 0.4%Triton X-100（in PBS）
- 0.1%Triton X-100（in PBS with 5 mg/mL BSA）
- 4% Paraformaldehyde (in PBS)
- Immunohistochemistry (IHC) pen
- Dewaxing reagents (for paraffin sections)
- Reagents for paraffin section processing
- Anti-fluorescence quenching mounting medium
- ddH2O

Experimental Design

A. Positive Control

Use DNase I to generate positive control slides. DNase I digests DNA to expose 3'-OH ends, simulating apoptosis. One control per experiment is sufficient (to verify the effectiveness of the reagents and procedures).

B. Negative Control

Use TUNEL Reaction Buffer without TdT enzyme (replace TdT with ddH_2O). This control helps exclude non-specific staining due to background apoptosis or procedural artifacts and is also used to adjust imaging exposure.

C. Experimental Group

Follow the standard protocol described below.

Protocol

1. Sample Preparation

(1) Adherent Cells or Cell Smears

a. Rinse once with PBS.

Note: If cells are loosely attached, allow the smear to dry to improve adhesion.

b. Fix with 4% paraformaldehyde (in PBS) at 4°C for 30 min. Rinse twice with PBS.

c. Permeabilize with 0.4% Triton X-100 (in PBS) at room temperature (RT) for 20 min. Rinse twice with PBS.
d. Proceed to Step 2: TUNEL Reaction.
(2) Suspension Cells or Cell Suspensions
a. Collect 3–5×10^6 cells, centrifuge at 1000 rpm for 5 min, and rinse twice with PBS.
b. Fix with 4% paraformaldehyde (in PBS), resuspend cells thoroughly, and incubate at 4°C for 30 min. Centrifuge at 2000 rpm for 5 min, rinse twice with PBS.
c. Permeabilize with 0.4% Triton X-100 (in PBS) at RT for 20 min. Centrifuge and rinse twice with PBS.
d. Proceed to Step 2: TUNEL Reaction.
(3) Paraffin-Embedded Tissue Sections
a. Place sections on slides and bake at 60°C for 60 min.
b. Deparaffinize and rehydrate:
Xylene I (10 min) → Xylene II (10 min) → 100% Ethanol I (5 min) → 100% Ethanol II (5 min) → 95% (5 min) → 90% (5 min) → 80% (5 min) → 70% (5 min) → ddH_2O rinse (5 min ×2)
Note: Xylene is toxic and volatile. Perform in a fume hood.
c. Dry around the tissue with filter paper. Outline the tissue with an IHC pen.
d. Permeabilize: Dilute Proteinase K (2 mg/mL) 1:50 in PBS to a final concentration of 40 μg/mL. Add 100 μL per sample and incubate at 37°C for 30 min.
Note: Optimize concentration and incubation time for different tissue types.
e. Rinse 3× with PBS (5 min each). Remove excess liquid and place slides in a humid chamber.
f. Proceed to Step 2: TUNEL Reaction.
(4) Frozen Tissue Sections
a. Bring sections to RT. Fix in 4% paraformaldehyde (in PBS) at RT for 30 min. Rinse 3× with PBS (10 min each).
Optional: After fixation, rinse with 2 mg/mL glycine for 10 min to neutralize residual fixative.
b. Dry and outline the tissue as above.
c. Permeabilize with Proteinase K (40 μg/mL) at 37°C for 20 min.
Note: If staining is still weak, use 1% Triton X-100 (in PBS) for 3–5 min instead.
d. Rinse 3× with PBS and place in humid chamber.
e. Proceed to Step 2: TUNEL Reaction.
(5) Positive Control Treatment (Positive Control Only)
a. Dilute 10× DNase I Buffer to 1× with ddH_2O.
b. Add 100 μL 1× buffer to sample and incubate at RT for 5 min.
c. Dilute DNase I (2 U/μL) 1:100 in 1× buffer to make 20 U/mL working solution.

d. Remove buffer, add 100 µL DNase I working solution, and incubate at RT for 15 min.
e. Rinse twice with PBS.
f. Proceed to Step 2: TUNEL Reaction.

2. TUNEL Reaction

Reagent	1 Sample	5 Sample	10 Sample
TdT Enzyme	2 µL	10 µL	20 µL
Fluorescein 555 TUNEL Reaction Buffer	48 µL	240 µL	480 µL
TUNEL Total Volume	50 µL	250 µL	500 µL

Prepare TUNEL reaction mix fresh before use.
(1) Adherent Cells, Smears, or Tissue Sections
a. Add 50 µL TUNEL reaction mix per sample. Cover evenly and incubate at 37°C in the dark (15–30 min for cells, 1 h for tissues).
Tip: Use anti-evaporation film or a cut plastic cover to prevent drying.
b. Remove reaction mix, rinse twice with PBS, then wash 3× with 0.1% Triton X-100 (in PBS with 5mg/mL BSA), 5 min each.
c. Optional: Stain with DAPI (5 µg/mL) for 5 min at RT. Rinse twice with PBS.
d. Optional: Mount with anti-fluorescence quenching medium. Add 20 µL per sample, cover with coverslip, and gently tap to remove bubbles.
e. Keep sample moist with PBS and observe under a fluorescence microscope immediately.
(2) Suspension Cells
a. Add 50 µL TUNEL reaction mix per tube, gently resuspend cells, and incubate at 37°C in the dark for 15–30 min. Gently mix every 10 min.
b. Centrifuge at 2000 rpm for 5 min, remove supernatant, and wash 2× with 0.1% Triton X-100 (in PBS with 5 mg/mL BSA), 5 min each.
c. Stain with DAPI (5 µg/mL) for 5 min.
d. Resuspend in 400 µL PBS and analyze by flow cytometry or prepare smears for microscopy.

Notes

1. Briefly centrifuge all reagents before use.
2. If background is high or non-specific staining occurs, reduce incubation time.
3. Always include positive and negative controls.

4. Wear gloves and mask when handling Component A. If skin contact occurs, rinse immediately with plenty of water.
5. Protect samples from light to prevent fluorescence quenching.
6. For research use only. Not for diagnostic or therapeutic use.
7. Wear lab coat and disposable gloves for safety.

This product is for research use only!