

## One-step TUNEL Apoptosis Assay Kit (Red Fluorescence)

Cat #: D-AKK2011

Size: 50T

Storage: Stored at -20°C for 6 months, protected from light

### Assay Principle

One of the most easily measured features of apoptotic cells is the break-up of the genomic DNA by cellular nucleases. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is a method for detecting DNA fragmentation by labeling the 3'-hydroxyl termini in the double-strand DNA breaks generated during apoptosis. The TUNEL assay relies on the presence of nicks in the DNA which can be identified by TdT, an enzyme that catalyzes the addition of dUTPs that are labeled with fluorescein. This kit provides all the essential components with an optimized assay protocol, suitable for fluorescence microplate reader, fluorescence microscope, or flow cytometer. Its signal can be easily detected at the popular Red channel (Ex/Em=555 nm/565 nm)

### Materials Supplied and Storage Conditions

Kit components	Size (50T)	Storage conditions
TdT Enzyme	50 µL	-20°C
Equilibration Buffer (5×)	1 mL	-20°C
Label Mix Red	250 µL	-20°C, protected from light
DAPI (500×)	12 µL	-20°C, protected from light
BSA Working Solution	15 mL	-20°C
TritonX-100 (100%)	100 µL	4°C
DNase I (5 U/µL)	10 µL	-20°C

## Materials Required but Not Supplied

- Centrifuge, fluorescence microscope
- 96-well cell culture plate, precision pipettes, disposable pipette tips, phosphate-buffered saline (PBS, pH 7.4)
- 4% paraformaldehyde, deionized water, proteinase K, tissue spontaneous fluorescence quenching agent

## Reagent Preparation

**DAPI (1×):** According to the actual consumption, dilute DAPI (500×) to DAPI (1×) with PBS.

**TritonX-100 (0.3%):** According to the actual consumption, dilute 100% TritonX-100 to 0.3% TritonX-100 with PBS.

**1×Equilibration Buffer:** According to the actual consumption, dilute 5×Equilibration Buffer to 1×Equilibration Buffer with deionized water.

## Assay Procedure

### A. Sample Preparation

#### 1. For adherent cells (Analysis by Fluorescence microscope)

- (1) Grown in a 96-well microplate culture for at least 24 h. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
- (2) Remove the medium and fix the cells with 50  $\mu$ L 4% paraformaldehyde for 30 min at room temperature.
- (3) Remove the fixation solution and wash with 200  $\mu$ L PBS 3 times (5 min each time).
- (4) Add 50  $\mu$ L 0.3% Triton X-100 after the fixation, and incubate the plate for 30 min at room temperature.
- (5) Wash the cells with 50  $\mu$ L BSA Working Solution 3 times. (Proceed with Step B.1)

**Optional: For cell climbing and other pore plate cells, the volume of fixative and permeating agent can be adjusted according to the actual situation.**

#### 2. For non-adherent cells (Analysis by Flow Cytometry)

- (1) Culture cells to an optimal density (about 1 to  $2 \times 10^6$  cells/mL). Induce apoptosis by desired methods. Concurrently incubate a control culture without induction.
- (2) Collect  $1-5 \times 10^6$  cells by centrifugation at 300 g. Wash with 0.5 mL of PBS twice.
- (3) Add 1 mL of 4% paraformaldehyde and incubate on ice for 30 min.

- (4) Centrifuge the cells at 300 g. Remove the supernatant and resuspend in 1 mL PBS. Repeat this wash twice.
- (5) Resuspend cells in 500  $\mu$ L 0.3% Triton-X 100 for 5 min at room temperature to permeabilize (Alternatively, resuspend the cells in 100  $\mu$ g/mL Proteinase K for 5 min to permeabilize).
- (6) Centrifuge the cells at 300 g. Remove the supernatant and resuspend in 1 mL PBS. Repeat this wash twice and Proceed with Step B.2.

### **3. For Paraffin-Embedded Tissue (Analysis by Fluorescence microscope)**

- (1) Deparaffinize tissue by immersing twice in xylene for 10-20 min.
- (2) Rehydrate tissue by the following washes (in the order given): two washes for 5 min each in 100% ethanol, then one wash for 3 min each successively in 95%, 70%, and 50% ethanol.
- (3) Wash the sample in 200-500  $\mu$ L PBS twice for 5 min each.
- (4) Drain excess PBS from tissue and incubate for 15 min in 20  $\mu$ g/mL Proteinase K (in PBS, preparation before use) solution.

**Note: The time of protease digestion will have to be optimized for specific tissue types and thicknesses. Over digestion by protease will result in loss of cellular structure and possible release of tissue section from slide. Under digestion will result in poor TdT labeling.**

- (5) Terminate the protease treatment by washing cells three times for 5 min each in PBS with gentle agitation. Proceed with Step B.1.

### **4. For Frozen tissue sections (Analysis by Fluorescence microscope)**

- (1) After sections have dried on the slide, fix with 200  $\mu$ L 4% paraformaldehyde for 30min at room temperature.
- (2) Wash by immersing in 200-500  $\mu$ L PBS twice for 5 min each.
- (3) Drain excess PBS from tissue and incubate for 15 min in 20  $\mu$ g/mL Proteinase K (in PBS) solution.
- (4) Terminate the protease treatment by washing cells three times for 5 min each in 200-500  $\mu$ L PBS with gentle agitation. Proceed with Step B.1.

**Note: 1. Tissue sections will produce spontaneous fluorescence, which can be treated with tissue spontaneous fluorescence quenching agent. 2. Setting of positive control (optional), after step A is completed, cells or tissues can be digested with 10 U/mL DNase I (dilute 5 U/ $\mu$ L to 10 U/mL with PBS) at room temperature for 10-20 min, and then analyzed with fluorescence microscope.**

## B. TUNEL assay

### 1. Analysis by Fluorescence microscope

(1) Prepare TdT labeling reaction buffer just before use based on the number of samples to be assayed:

Reaction Components	Volume Per Well ( $\mu\text{L}$ )
TdT Enzyme	1
Equilibration Buffer (5 $\times$ )	10
Label Mix Red	5
Deionized Water	34
Total Volume	50

**Note: Before preparing TdT-labeled reaction buffer, rewarm each component to room temperature. The Equilibration Buffer (5 $\times$ ) stock solution is stored at low temperature, resulting in a small amount of component precipitation. Please invert and mix before use. The Equilibration Buffer (5 $\times$ ) contains cacodylate and cobalt chloride, highly toxic chemicals. After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately. Do not drink, eat or smoke when using.**

(2) Add 50  $\mu\text{L}$  of the reaction mixture (from Step A.1, 3 and 4) to each sample (It is recommended 50  $\mu\text{L}$  for 96-well plates, 100-200  $\mu\text{L}$  for 24-well plates, Tissue sections is recommended to add 100-200  $\mu\text{L}$  covering tissue) and incubate at 37°C for 2 h (this time should be different depending on the samples ) in a humidified box.

(3) Wash samples 3 times for 5 min each in PBS.

(4) Counterstain sample by incubating in 1 $\times$ DAPI in PBS for 10 min.

**Note: If you need to calculate the proportion of apoptotic cells, overstaining is recommended. Concentration of counterstain may have to be adjusted depending on the tissue being stained. Overstaining by DAPI may result in difficulty in observing the fluorescein label.**

(5) Wash sample 3 times for 5 min each in PBS.

(6) For cell slides, paraffin sections and frozen section samples, add an aqueous mounting medium or an antifade solution, mount a coverslip and analyze using fluorescent microscopy with a fluorescein filter. For cell samples in well plates and petri dishes, add appropriate amount of PBS to immerse the cells, then take pictures and observe with a fluorescence microscope. Its signal can be easily detected at the popular Red channel (Ex/Em=555 nm/565 nm).

### 2. Analysis by Flow Cytometry

- (1) Resuspend cells in 100  $\mu$ L of 1 $\times$ Equilibration Buffer. Incubate at room temperature for 10 min.
- (2) Centrifuge cells at 300 g. Remove the supernatant and resuspend in 50  $\mu$ L of TdT labeling reaction buffer. Incubate at 37°C for 2 h (the incubation time should be different depending on the samples ), during which periodically mix cells gently.
- (3) Centrifuge cells at 300 g. Remove the supernatant and resuspend in 1 mL PBS. Repeat wash twice.
- (4) Resuspend in 200  $\mu$ L 1 $\times$ DAPI in PBS. Incubate 10 min.
- (5) Analyze cells by flow cytometry.

## FAQs

1. Is antigen repair necessary for TUNEL apoptosis detection?

Cell and tissue sample need to be fixed first, no antigen repair is required, and this kit marks broken genes in the nucleus, not antigen proteins.

2. Can TUNEL assay be double-dyed with immunofluorescence (IF)? In what order?

It can be co-dyed with IF. It is recommended to do TUNEL test first and then do IF.

3. Can this product replace acridine red dyeing kit?

Yes, acridine red staining kit components are carcinogenic, so it is safer to use the TUNEL kit for apoptosis detection.

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.