TUNEL Apoptosis Detection Kit (Green Fluorescence)

Cat #: orb1147899 (manual)

Product name: TUNEL Apoptosis Detection Kit (Green Fluorescence)

Catalog number: orb1147899

Applicable samples: Flow cytometry and fluorescence detection of cell and tissue samples

Storage: Stored at -20℃ 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 FITC channel (Ex/Em=490 nm/520 nm).

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	100 T	
TdT Enzyme	50 µL	100 µL	-20℃
Equilibration Buffer (5×)	1 mL	2 mL	-20℃
Label Mix Green	17 µL	35 µL	-20℃, protected from light
Probe Diluent	0.5 mL	1 mL	4℃
DAPI (500×)	12 µL	24 µL	-20℃, protected from light
BSA Working Solution	15 mL	30 mL	-20℃
TritonX-100 (100%)	100 µL	100 µL	4℃
DNase I (5 U/µL)	10 µL	10 µL	-20℃
Proteinase K (20mg/mL)	10 µL	20 µL	-20℃

Materials Required but Not Supplied

- Centrifuge, fluorescence microscope
- 96-well cell culture plate, precision pipettes, disposable pipette tips, 10mM Tris pH7.5, phosphate-buffered saline (PBS, pH 7.4)
- 4% paraformaldehyde, deionized water, tissue spontaneous fluorescence quenching agent

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Reagent Preparation

Working Label Mix Green: According to the actual consumption, dilute Label Mix Green 15 times with Probe Diulent to obtain Working Label Mix Green, the unused Working Label Mix Green can also be stored at -20℃ and protected from light for 3 months after aliquoting to avoid repeated freezing and thawing.

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:** Analysis by Flow Cytometry requires the preparation of 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 µL 4% paraformaldehyde for 30 min at room temperature.

(3) Remove the fixation solution and wash with 200 µL PBS 3 times (5 min each time).

(4) Add 50 µL 0.3% Triton X-100, after the fixation, and incubate the plate for 30 min at room temperature.

(5) Wash the cells with 50 µL 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 2×106 cells/mL). Induce apoptosis by desired methods. Concurrently incubate a control culture without induction.

(2) Collect 1-5×106 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 µL 0.3% Triton-X 100 for 5 min at room temperature to permeabilize (Alternatively, resuspend the cells in 100 µg/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 µL PBS twice for 5 min each.

(4) Drain excess PBS from tissue and incubate for 15 min at room temperature in 20 µg/mL Proteinase K (in 10mM Tris pH7.5, 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 µL 4% paraformaldehyde for 30 min at room temperature.

(2) Wash by immersing in 200-500 µL PBS twice for 5 min each.

(3) Drain excess PBS from tissue and incubate for 15 min at room temperature in 20 µg/mL Proteinase K (in 10mM Tris pH7.5) solution.

(4) Terminate the protease treatment by washing cells three times for 5 min each in 200-500 µL 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/µL 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 (µL)
TdT Enzyme	1
Equilibration Buffer (5×)	10
Working Label Mix Green	5
Deionized Water	34
Total Volume	50

Note: Before preparing TdT-labeled reaction buffer, rewarm each component to room temperature. The Equilibration Buffer (5×) 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×) 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 µL of the reaction mixture (from Step A.1, 3 and 4) to each sample (It is recommended 50 µL for 96-well plates, 100-200 µL for 24-well plates, Tissue sections is recommended to add 100-200 µL 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×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 FITC channel (Ex/Em=490 nm/520 nm).

Microscopy photography tips: 1) Use the DAPI channel to locate cell positions; 2) Prioritize negative control group imaging, as the fluorescence of the negative control is non-specific staining fluorescence that needs to be subtracted to no fluorescence through black and white balance; 3) When taking photos of the experimental or positive group, subtract the same black and white balance as the negative control to obtain the effective fluorescence of the experimental or positive group.

2. Analysis by Flow Cytometry

(1) Resuspend cells in 100 µL of 1×Equilibration Buffer. Incubate at room temperature for 10 min.

(2) Centrifuge cells at 300 g. Remove the supernatant and resuspend in 50 µL of TdT labeling reaction buffer. Incubate at 37℃ 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 µL 1×DAPI in PBS. Incubate 10 min.

(5) Analyze cells by flow cytometry.

Highlight moment: In addition to detecting apoptosis based on changes in nucleus, changes in cytoplasm and mitochondria can also reflect apoptosis.

FAQ

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 orange dyeing kit?

   Yes, acridine orange 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.

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