TUNEL Apoptosis Detection Kit (Green Fluorescence)

Cat #: KTA2010 Size: 50 T/100 T

	TUNEL Apoptosis Detection Kit (Green Fluorescence)		
REF	**Cat #:** KTA2010	LOT	**Lot #:** Refer to product label
	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	250 μL	500 μL	-20℃, protected from light
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℃

Materials Required but Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Phosphate-buffered saline (PBS)
- Fluorescence microscope

- 4% paraformaldehyde (in PBS, pH 7.4)
- Proteinase K

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 in PBS 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 permeabilization reagent (0.3% Triton X-100 in PBS, not supplied) 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 slide and 24-well plate cells, the volume of fixation solution and permeabilization reagent could be adjusted according to the actual situation. You may also prepare a positive control for Tunel reaction using by digesting cells with 10 U/mL DNAase I for 10 min at room temperature before proceeding to Tunel reaction (Step B.1).

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

(1) Culture cells to an optimal density (about 1 to 2×10^6 cells/mL). Induce apoptosis by desired methods. Concurrently incubate a control culture without induction.

(2) Collect 1-5×10^6 cells by centrifugation at 300 g. Wash with 0.5 mL of PBS twice.

(3) Add 1 mL of 4% paraformaldehyde (in PBS, pH 7.4) 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 in 20 µg/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 µL 4% paraformaldehyde in PBS 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 in 20 µg/mL Proteinase K (in PBS) 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.

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
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℃ 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).

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.

Recommended Products

Catalog No.	Product Name
KTA0002	Annexin V-AbFluor™ 488 Apoptosis Detection kit (Green Fluorescence)
KTA2011	TUNEL Apoptosis Detection Kit (Orange Fluorescence)
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)
KTD102-EN	Apoptosis Assay Cocktail

Disclaimer

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