One-Step TUNEL Apoptosis Assay Kit (Green Fluorescence)

Cat #: KTA2010 Size: 20 T/50 T/100 T

	One-Step TUNEL Apoptosis Assay 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 12 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
	20 T	50 T	100 T	
TdT Enzyme	40 μL	100 μL	200 μL	-20℃
Equilibration Buffer (5×)	0.8 mL	1 mL	2 mL	-20℃
Label Mix Green	4 μL	17 μL	35 μL	-20℃, protected from light
Probe Diluent	0.2 mL	0.5 mL	1 mL	-20℃
DAPI (500×)	5 μL	12 μL	24 μL	-20℃, protected from light
BSA Working Solution	6 mL	15 mL	30 mL	-20℃
TritonX-100 (100%)	100 μL	100 μL	100 μL	4℃
DNase I (5 U/μL)	4 μL	10 μL	10 μL	-20℃
10×DNase I Buffer	0.3 mL	0.6 mL	0.6 mL	-20℃
Proteinase K (20mg/mL)	4 μL	10 μL	20 μL	-20℃
Tissue Autofluorescence Quencher	2 mL	5 mL	10 mL	RT, protected from light

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, ylene, ethanol, water-based antifade mounting medium

Reagent Preparation

Working Label Mix Green: According to the actual consumption, dilute Label Mix Green 25 times with Probe Diluent 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: According to the actual consumption, dilute 5×Equilibration Buffer to 1×Equilibration Buffer with deionized water, 1×Equilibration Buffer was used to equilibrate samples before Tunel labeling.

1×DNase I Buffer: According to the actual consumption, dilute 10×DNase I Buffer to 1×DNase I Buffer with deionized water.

Tissue Autofluorescence Quencher: Ready to use as supplied. Equilibrate to room temperature before use. It can significantly quench the autofluorescence caused by lipofuscin. Tighten the bottle cap after use to prevent evaporation.

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)

Note:

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

2) An alternative positive control can be prepared by treating the sample with DNase I to induce DNA fragmentation after step A.1.5. Setting of positive control, after sample preparation, cells or tissues can be digested with 10 U/mL DNase I (dilute 5 U/µL to 10 U/mL with 1×DNase I Buffer) at room temperature for 10-20 min, the sample is washed 3 times with PBS for 5 min each time, and then analyzed with fluorescence microscope. If it is a tissue section sample, it is recommended to stain and wash the positive control in a separate staining jar for subsequent operations.

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 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.

Note: An alternative positive control can be prepared by treating the sample with DNase I to induce DNA fragmentation after step A.2.6. The detailed steps of DNase I treatment can refer to the precautions 2) in point 1 of A.1.5 for the treatment of adherent cell samples.

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.

Note: It can also be operated according to the tissue dewaxing and renaturation operation system of your own laboratory.

(3) Wash the sample in 200-500 µL PBS twice for 5 min each.

(4) Drain excess PBS from tissue, after the sections were shaken dry, circle the tissue with a Pap Pen, and incubate for 15 min at room temperature 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.

Note: An alternative positive control can be prepared by treating the sample with DNase I to induce DNA fragmentation after step A.3.5. The detailed steps of DNase I treatment can refer to the precautions 2) in point 1 of A.1.5 for the treatment of adherent cell samples.

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, after the sections were shaken dry, circle the tissue with a Pap Pen, and incubate for 15 min at room temperature in 20 µg/mL Proteinase K (in PBS, preparation before use) 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: An alternative positive control can be prepared by treating the sample with DNase I to induce DNA fragmentation after step A.4.4. The detailed steps of DNase I treatment can refer to the precautions 2) in point 1 of A.1.5 for the treatment of adherent cell samples.

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	2
Equilibration Buffer (5×)	10
Working Label Mix Green	5
Deionized Water	33
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 1×Equilibration Buffer (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 RT for 5 min, protected from light.

(3) Drain excess 1×Equilibration Buffer, then 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 1-2 h (this time should be different depending on the samples) in a humidified box.

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

(5) Counterstain sample by incubating in 1×DAPI in PBS for 10 min.

Note: If you need to calculate the proportion of apoptotic cells, it is recommended to counterstain with DAPI. 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.

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

(7) Quench the Autofluorescence of the Tissue. For tissue samples, add Tissue Autofluorescence Quencher, incubate at room temperature for 5 min, protected from light. Soak in deionized water for 5 min, and then soak in PBS 3 times for 5 min each time.

Note: 1. Tissue sections will produce spontaneous fluorescence, and only tissue samples need to quench the spontaneous fluorescence. After Tissue Autofluorescence Quencher treatment, the tissue will be stained blue, and the fluorescence intensity of Tunel will be reduced to some extent, but the fluorescence microscope photography will not be affected; 2. The incubation time of the Tissue Autofluorescence Quencher needs to be precisely determined. It can be timed for 5 minutes after adding the Tissue autofluorescence Quencher to the first sample. 3.Tissue Autofluorescence Quencher is volatile. During the incubation process, it needs to be replenished in time to prevent the reagent from drying out, solidifying and caking, which may affect the subsequent photography and observation.

(8) 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 1-2 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.

Typical Data

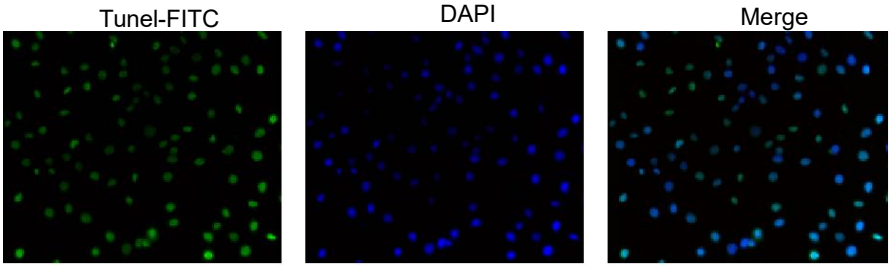

Figure 1. Apoptosis detection effect diagram of Hela cells after one-step staining with TUNEL Apoptosis Detection Kit (Green Fluorescence).

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.

4. The tissue sample has no positive information number?

(1) Increase the working concentration of protease K;

(2) When conducting TUNEL analysis (i.e., before staining and incubation), equilibrate once with 1x Equilibration Buffer and then add the staining working solution.

(3) During the experiment, be careful not to dry the plate, as it will increase the background signal.

5. For the PBS cleaning step in the experimental process, should it be completed on a shaker or should it be still immersed in PBS?

Both are OK. When cleaning the shaker, set it to a low speed and do it gently.

6. What steps should be noted during the experiment to reduce the non-specific fluorescence of the tissue?

(1) Tissue autofluorescence quenchers can be used. First, capture the negative group and subtract the non-specific fluorescence.

(2) The use of acidic or alkaline fixatives can cause DNA damage and result in false positives. It is recommended to use a ph-neutral fixative to fix tissues or cells.

(3) Excessively high concentration of the fixative or prolonged duration can lead to autolysis of cells, irregular breakage of DNA strands, and false positivity. It is recommended to use 4% paraformaldehyde solution.

(4) If the treatment time of proteinase K is too long or too large, it will damage the structure of nucleic acids and cause false positives. You can explore the concentration and time.

(5) If the TUNEL staining time is too long, the incubation time can be reduced.

(6) If the sample is not thoroughly washed, the number of PBS washes after staining can be increased to 5 times to avoid non-specific staining of the sections.

7. What is the state of thorough dewaxing like?

When the tissue is transparent and no water adheres to the slide, it indicates that the dewaxing is clean.

8. Can neutral gum be used for sealing tissue sections?

Neutral gum cannot be used because after the section staining is completed, it is in a water-soluble state. Water-soluble mounting agent should be used. Neutral gum is a non-water-soluble mounting agent. After mounting, it will stick together and the section will be damaged. Antifade mounting medium agent can be used for mounting.

9. What is the recommended thickness for frozen sections?

The general thickness of frozen sections is between 8 and 10 μm.

Recommended Products

Catalog No.	Product Name
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit
KTA2011	One-Step TUNEL Apoptosis Assay Kit (Red Fluorescence)
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)

KTA3020	Caspase-1 Assay Kit (Colorimetric)
KTA3022	Caspase-3 Assay Kit (Colorimetric)
BMU104	SuperKine™ Enhanced Antifade Mounting Medium

Disclaimer

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