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Protocol
Cat. No. K1133

One-step TUNEL FITC Apoptosis Detection Kit

Introduce

The One-step TUNEL FITC Apoptosis Detection Kit can be used to detect breaks in nuclear DNA in tissues or cells during apoptosis. Intracellular DNA endonucleases are activated during apoptosis, cutting off genomic DNA between nucleosomes, and DNA degrades into 180 - 200 bp or other integer multiple fragments. The 3´-OH terminal produced by the genomic DNA break is added to the end with the labeled dUTP at its end under the action of terminal Deoxynucleotidyl Transferase (TdT), This allows detection under a fluorescence microscope or flow cytometry (Ex/Em=429nm/517nm).

This kit can be used in a wide range of applications to detect apoptosis in frozen or paraffin sections, as well as to detect apoptosis of cultured adherent or suspension cells.

Materials/Composition

Component	20 rxns	50 rxns	100 rxns
5 × Equilibration Buffer	1 ml	2 × 1 ml	4 × 1 ml
FITC-12-dUTP Labeling Mix	100 μl	250 μl	2 × 250 μl
TdT Enzyme	20 μl	50 μl	2 × 50 μl
Proteinase K (2 mg/ml)	40 μl	100 μl	2 × 100 μl
DNase I (2 U/μl)	5 μl	13 μl	25 μl
10 × DNase I Buffer	100 μl	260 μl	500 μl

Store conditions

Place product at -20 °C for storage, stable for 1 year; Among them, FITC-12-dUTP Labeling Mix, stored at -20 °C

protected from light.

Scope of application

Apoptosis detection of paraffin sections, frozen sections, adherents, and suspension cultured cells

Materials Not Supplied

Reagents: 4% paraformaldehyde, DAPI/PI, Triton X-100, PBS, xylene (paraffin section), absolute ethanol (paraffin section), ddH2O, Sealing solution containing anti-fluorescent quenching agent (recommended).

Other materials: cell crawling/slides, wet cassettes.

Experimental protocol

NO.1: Experimental procedure of tissue sectioning/adherent cells

- Paraffin-embedded tissue sections

1. Sample pretreatment

   1.1 Immerse the slices in xylene dewaxing 2 times (5- 10min/per time) at room temperature to completely remove the paraffin. (Xylene is toxic and volatile, so the operation should be in a separate laboratory or fume hood.)

   1.2 Immerse the slices in absolute ethanol, soaking and rinsing twice for 5 min each at room temperature.

   1.3 Immerse the slices in 90%, 80%, and 70% ethanol for 3 min each at room temperature.

   1.4 Soak and wash the slides with 1× PBS and carefully drain excess liquid around the sample on the slides with filter paper.

   1.5 Dilute Proteinase K solution at a concentration of 2 mg/ml with 1× PBS at a ratio of 1:100 to a final concentration of 20 μg/ml. Add 100 μl of diluted Proteinase K solution dropwise to each sample to cover the entire sample area and incubate for 20 min at room temperature.

   Note: Reaction time may vary from tissue or species to species. Too long permeability time for Proteinase K may cause tissue sections to fall off the slide *in subsequent steps, which is too short and causes insufficient permeability treatment and affects labeling efficiency. It is recommended to perform a pre-experiment to determine the reaction time.*

   1.6 Soak and wash the samples 3 times with 1×PBS solution for 5 min each. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. The treated sample is kept moist in a wet box.

   Note: This step must be done by cleaning the proteinase K, otherwise it will seriously

interfere with the subsequent labeling reaction.

2. (Optional step). Positive control-DNase I treatment (Only positive controls perform this step, other samples go directly to step 3. Marking and detection).

   2.1 Dilute 10× DNase I Buffer to 1× DNase I Buffer for backup with ddH2O at a ratio of 1:10.

   2.2 Add 100 µl of 1× DNase I Buffer dropwise onto the permeable sample and equilibrate at room temperature for 5 min.

   2.3 Dilute DNase I (2 000 U/ml) with 1×DNase I Buffer to a final concentration of 20 U/ml.

   2.4 Gently aspirate the excess liquid, add 100 µl of each sample dropwise to the 20 U/ml DNase I and incubate at room temperature for 10 min.

   2.5 Gently aspirate the excess liquid and thoroughly soak and rinse the tissue sections in a staining vat containing 1×PBS for 2-3 times.

   Note: Positive control slides must use a separate staining jar. Residual DNase I on positive control slides may create false positive false signals in the experimental group.

3. Marking and detection

   3.1 Dilute 5 × Equilibration Buffer to 1 × Equilibration Buffer with ddH2O at a ratio of 1:5.

   3.2 Each sample is drizzled with 100 µl of the × Equilibration Buffer to cover all areas of the sample to be examined, equilibrated at room temperature for 10 – 30 min.

   3.3 During equilibration, the labeling fluid is prepared according to the following table under light-protected conditions.

Component	Negative control	Positive control/sample
ddH_2O	35 µl	34 µl
5 × Equilibration Buffer	10 µl	10 µl
FITC-12-dUTP Labeling Mix	5 µl	5 µl
TdT Enzyme	0 µl	1 µl

   3.4 After equilibration is complete, aspirate the 1 × Equilibration Buffer with absorbent

paper, then add 50 µl of labeled reaction solution to the sample dropwise, taking care to avoid light.

Note: Labeled liquid volume: For reactions with an area less than 5 cm^2, the required volume is 50 µl, multiply 50 µl by the number of experimental and positive control reactions to determine the total volume of TdT labeled reaction solution required. *For samples with a larger surface area, the reagent volume can be increased proportionally.*

3.5 Place a paper towel soaked in water on the bottom of the black wet box protected from light. Place the slides inside a wet cassette and incubate at 37 °C for 60 min.

3.6 Gently remove excess liquid and wash 2 times with fresh 1 × PBS solution for 5 min each room temperature.

Note: If the background is too high, to reduce the background, the sample can be washed 3 times with PBS containing 0.1% Triton X-100 and 5 mg/ml BSA for 5 min after washing with 1 × PBS, which can clear the free unreacted markers.

3.7 Gently wipe off the 1× PBS solution around and on the back of the sample with filter paper.

3.8 The samples were counterstained in the dark with a 1 µg/ml PI solution freshly prepared with 1 × PBS or a DAPI solution freshly prepared with 1 × PBS at a concentration of 2µg/ml, leaving the stain at room temperature for 5 min.

3.9 Wash the samples and immerse the slides in 1 × PBS solution 3 times for 5 min each room temperature.

3.10 Gently aspirate excess liquid and add an appropriate amount of anti-fluorescence quenching agent to the sample area to keep the sample moist and seal.

3.11 Analyze the sample immediately under a fluorescence microscope and observe the green fluorescence at 520 ± 20 nm fluorescence; Observe the red fluorescence of the PI at >620nm, or the blue fluorescence of DAPI at 460 nm.

■ **Frozen tissue sections**

4. Sample pretreatment

4.1 Place the frozen slices on a long rack and dry at room temperature for 20 min.

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https://www.apexbt.com/; Email: sales@apexbt.com.

4.2 Immerse the slides in 4% paraformaldehyde solution (dissolved in 1×PBS) and fix at room temperature for 30 min.

4.3 Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper.

4.4 Immerse the slides in 1× PBS solution and wash twice for 5 min each.

4.5 Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper.

4.6 Prepare Triton X-100 solution with 1×PBS at a concentration of 0.2%. Add 100 μl of TritonX-100 solution dropwise to each sample to cover the entire sample area and incubate at room temperature for 15 min. If the permeability effect is not good, dilute Proteinase K solution with 1× PBS at a concentration of 2 mg/ml at a final concentration of 20 μg/ml at a ratio of 1:100. Add 100 μl of diluted Proteinase K solution dropwise to each sample so that the solution covers the entire sample area and incubate for 10 min at room temperature.

Note: Permeability time is too long to increase the risk of tissue sections falling off the slide during subsequent wash steps, and too short may result in inadequate permeation treatment, affecting labeling efficiency. For best results, incubation times may need to be optimized.

4.7 Wash the samples 2-3 times with 1× PBS solution for 5 min each. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. The treated sample is kept moist in a wet box.

5. (Optional step) Positive control-DNase I treatment (only positive controls perform this step, other samples go directly to step 6. Labeling and detection).

5.1. Dilute 10× DNase I Buffer to 1× DNase I Buffer for backup with ddH2O at a ratio of 1:10.

5.2 Add 100 μl of 1× DNase I Buffer dropwise onto the permeable sample and equilibrate at room temperature for 5 min.

5.3 Dilute DNase I (2000 U/ml) with 1× DNase I Buffer to a final concentration of 20 U/ml.

5.4 Gently aspirate excess liquid, add 100 µl of each sample dropwise to 20 U/ml DNase I working solution and incubate at room temperature for 10 min.

5.5 Gently aspirate the excess liquid and thoroughly soak and wash the tissue sections 2-3 times in a staining jar filled with 1×PBS.

Note: Positive control slides must use a separate staining jar. Residual DNase I on positive control slides may create false positive false signals in the experimental group.

6. Marking and detection

6.1 Dilute 5 × Equilibration Buffer to 1 × Equilibration Buffer with ddH2O at a ratio of 1:5.

6.2 Each sample was drizzled with 100 µl of 1 × Equilibration Buffer to cover all areas of sample to be examined, equilibrated at room temperature for 10-30 min.

6.3 During equilibration, the labeling fluid is prepared according to the following table under light-protected conditions.

Component	Negative control	Positive control/sample
ddH_2O	35 µl	34 µl
5 × Equilibration Buffer	10 µl	10 µl
FITC-12-dUTP Labeling Mix	5 µl	5 µl
TdT Enzyme	0 µl	1 µl

6.4 After equilibration is complete, aspirate 1 × Equilibration Buffer with absorbent paper and then add 50 µl of labeled reaction solution to the sample dropwise , taking care to avoid light.

Note: Labeled liquid volume: For reactions with an area less than 5 cm^2, the required volume is 50 µl, multiply 50 µl by the number of experimental and positive control reactions to determine the total volume of TdT labeled reaction solution required. *For samples with a larger surface area, the reagent volume can be increased proportionally.*

6.5 Place a paper towel soaked in water on the bottom of the black wet box protected from light. Place the slides inside a wet cassette and incubate at 37 °C for 60 min.

6.6 Gently remove excess liquid and wash 2 times with fresh 1 × PBS solution for 5 min each

room temperature.

Note: If the background is too high, to reduce the background, the sample can be washed 3 times with PBS containing 0.1% Triton X-100 and 5 mg/ml BSA for 5 min after washing with 1 × PBS, which can clear the free unreacted markers.

6.7 Gently wipe off the 1× PBS solution around and on the back of the sample with filter paper.

6.8 The samples were counterstained in the dark with a 1 μg/ml PI solution freshly prepared with 1 × PBS or a DAPI solution freshly prepared with 1 × PBS at a concentration of 2 μg/ml, leaving the stain at room temperature for 5 min.

6.9 Wash the samples and immerse the slides in 1 × PBS solution 3 times for 5 min each room temperature.

6.10 Gently aspirate excess liquid and add an appropriate amount of anti-fluorescence quenching agent to the sample area to keep the sample moist and seal.

6.11 Analyze the sample immediately under a fluorescence microscope and observe the green fluorescence at 520 ± 20 nm fluorescence; Observe the red fluorescence of the PI at >620 nm, or the blue fluorescence of DAPI at 460 nm.

■ **Adherent cells**

7. Sample pretreatment

7.1 Preparation before the experiment:

- Preparation of cell crawlers: Culture adherent cells on Lab-Tek slide chambers or TC-treated cell crawlers. After apoptosis induction treatment, rinse slides 2 times with PBS for follow-up experiments.
- Preparation of cell smears: Resuspend cells at a concentration of 2×10^6 cells/ml at 1×PBS. Aspirate 50-100 μl of the cell suspension drops onto a polylysine-coated slide. Gently coat the cell suspension with a clean slide and enter into subsequent experiments.

7.2 Dip the crawler/smear into a staining jar filled with 4% paraformaldehyde (newly formulated at 1×PBS) and leave at 4 °C for 25 min for cell fixation.

7.3 Dip the crawler/smear in 1× PBS solution and wash twice for 5 min each at room temperature.

7.4 Gently remove excess liquid and carefully drain the liquid around the sample with filter paper.

7.5 Dilute Proteinase K solution at a concentration of 2 mg/ml with 1× PBS at a ratio of 1:100 to a final concentration of 20 μg/ml. Add 100 μl of diluted Proteinase K solution dropwise on each sample so that the solution covers the entire sample area and incubate at room temperature for 5 min; It can also be immersed in 0.2% Triton X-100 solution prepared with 1×PBS and incubated for 5 min at room temperature for permeability.

Note: Cell crawlers/smears are easier to remove during treatment, and it is recommended to use 0.2%-0.5% Triton X-100 solution for permeation to avoid despellation caused by permeation. Too long permeable proteinase K increases the risk of tissue sections falling off the slide during subsequent wash steps, and too short may result in inadequate permeation processing and affect labeling efficiency. For best results, it may be necessary to optimize the timing of Proteinase K incubation.

7.6 Wash the sample 2-3 times with 1× PBS solution. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. The treated sample is kept moist in a wet box.

8. (Optional step) Positive control-DNase I treatment (only positive controls perform this step, other samples go directly to step 9. Labeling and detection).

8.1. Dilute 10× DNase I Buffer to 1× DNase I Buffer for backup with ddH2O at 1:10 ratio

8.2 Add 100 μl of 1× DNase I Buffer dropwise onto the permeable sample and equilibrate at room temperature for 5 min.

8.3 Dilute DNase I (2000 U/ml) with 1× DNase I Buffer to a final concentration of 20 U/ml.

8.4 Gently aspirate excess liquid, add 100 μl of each sample dropwise to 20 U/ml DNase I working solution and incubate at room temperature for 10 min.

8.5 Gently aspirate excess liquid and soak the slides/crawlers thoroughly in a staining jar filled with 1×PBS 2-3 times.

Note: Positive control slides must use a separate staining jar. Residual DNase I on positive control slides may create false positive false signals in the experimental group.

9. Marking and detection

9.1 Dilute 5 × Equilibration Buffer to 1 × Equilibration Buffer with ddH2O at a ratio of 1:5.

9.2 Each sample was drizzled with 100 μl of 1 × Equilibration Buffer to cover all areas of sample to be examined, equilibrated at room temperature for 10-30 min.

9.3 During equilibration, the labeling fluid is prepared according to the following table under light-protected conditions.

Component	Negative control	Positive control/sample
ddH_2O	35 μl	34 μl
5 × Equilibration Buffer	10 μl	10 μl
FITC-12-dUTP Labeling Mix	5 μl	5 μl
TdT Enzyme	0 μl	1 μl

9.4 After equilibration is complete, aspirate 1 × Equilibration Buffer with absorbent paper and then add 50 μl of labeled reaction solution to the sample dropwise , taking care to avoid light.

Note: Labeled liquid volume: For reactions with an area less than 5 cm^2, the required volume is 50 μl, multiply 50 μl by the number of experimental and positive control reactions to determine the total volume of TdT labeled reaction solution required. *For samples with a larger surface area, the reagent volume can be increased proportionally.*

9.5 Place a paper towel soaked in water on the bottom of the black wet box protected from light. Place the slides inside a wet cassette and incubate at 37 °C for 60 min.

9.6 Gently remove excess liquid and wash 2 times with fresh 1× PBS solution for 5 min.

Note: If the background is too high, to reduce the background, the sample can be washed 3 times with PBS containing 0.1% Triton X-100 and 5 mg/ml BSA for 5 min after washing with 1 × PBS, which can clear the free unreacted markers.

9.7 Gently wipe off the 1× PBS solution around and on the back of the sample with filter

paper.

9.8 The samples were counterstained in the dark with a 1 μg/ml PI solution freshly prepared with 1 × PBS or a DAPI solution freshly prepared with 1 × PBS at a concentration of 2 μg/ml, leaving the stain at room temperature for 5 min.

9.9 Wash the samples and immerse the slides in 1 × PBS solution 3 times for 5 min each room temperature.

9.10 Gently aspirate excess liquid and add an appropriate amount of anti-fluorescence quenching agent to the sample area to keep the sample moist and seal.

9.11 Analyze the sample immediately under a fluorescence microscope and observe the green fluorescence at 520 ± 20 nm fluorescence; Observe the red fluorescence of the PI at >620 nm, or the blue fluorescence of DAPI at 460 nm.

NO.2: Experimental flow of suspension cells

1. (3-5) ×10^6cells are washed twice with 1×PBS and centrifuged at 1800 rpm (300× g) for 5 min each. Then resuspend with 0.5 ml 1× PBS.
2. Add 5 ml of paraformaldehyde solution prepared at 1× PBS concentration of 1% and incubate at 4 °C for 20 min for cell fixation.

   Note: To prevent cells from congregating into clumps, fixation can be performed while shaking slowly on a shaker.
3. Centrifuge at 4 °C at 1800 rpm (300× g) for 5 min, discard the supernatant and resuspend the cells with 5 ml 1×PBS.
4. Repeat the centrifugation and resuspend the cells with 0.5 ml 1× PBS.
5. Add 5 ml of 0.2% PBS solution of Triton X-100 for 5 min at room temperature, or add 5 ml of pre-chilled 70% ethanol on ice and incubate at -20 °C for 4 h.

   Note: The refractive index of cells after permeation will decrease, cell turbidity is not easy to observe, and careful manipulation should be taken during manipulation to avoid cell loss.
6. Centrifuge at 4 °C at 1800 rpm (300× g) for 5 min, discard the supernatant and resuspend the cells with 5 ml 1×PBS. Repeat the centrifugation and resuspend the cells with 1 ml 1×

PBS.

7. Transfer 2×10^6 cells to a new 1.5 ml centrifuge tube.
8. Centrifuge at 1800 rpm (300×g) for 5 min at 4 °C, discard the supernatant, and resuspend the cells with 100 µl 1× Equilibration Buffer (dilute 5×Equilibration Buffer in a 1:5 ratio with ddH2O). Incubate at room temperature for 5 min.
9. During cell equilibration, the labeling solution is prepared. The FITC-12-dUTP labeling mixture is melted on ice and the labeling solution is prepared according to the table below.

Component	Negative control	Positive control/sample
ddH_2O	35 µl	34 µl
5×Equilibration Buffer	10 µl	10 µl
FITC-12-dUTP Labeling Mix	5 µl	5 µl
TdT Enzyme	0 µl	1 µl

10. Centrifuge at 1800 rpm (300×g) for 5 min at 4 °C, discard the supernatant and resuspend the cells with 50 µl of TdT labeling solution. Incubate at 37 °C for 60 min at light-protected. Flick the tube wall every 15 min or gently resuspend the cells with a micropipette.

    Note: For one standard reaction of 2×10^6 cells, the required volume is 50 µl. Multiply 50 µl by the number of reactions above to determine the total volume of the desired TdT-labeled reaction fluid.
11. Add 1 ml of 20 mM EDTA to terminate the reaction and mix gently with a pipette.
12. Centrifuge at 1800 rpm (300× g) for 5 min at 4 °C, discard the supernatant, and resuspend cells with 1 ml of 0.1% TritonX-100 prepared in 1 × PBS containing 5 mg/ml BSA. Repeat once, wash twice.
13. Centrifuge at 1800 rpm (300× g) for 5 min at 4 °C, discard the supernatant, and resuspend the cells with 0.5 ml of 5 µg/ml PI solution prepared in 1×PBS containing 250 µg of DNase-free RNase A.
14. Incubate cells at room temperature for 30 min in the dark.
15. Analyze the cells with flow cytometry. Or observe the green fluorescence of FITC-12-dUTP with a standard fluorescence filtration device at 520±20 nm and the red

fluorescence of PI at >620 nm.

NO.3: TUNEL and immunofluorescence co-contamination experimental process

1. Paraffin section co-staining step

1.1 65 °C tablets for 30 min, xylene dewaxing, gradient alcohol hydration.

1.2 Antigen repair: The slices are placed in a citric acid repair solution of 0.1mol/L and pH = 6.0, heated for 6min to slight boiling in a microwave oven, and then maintained for 10min with medium and low fire, and naturally cooled for 20-30min after stopping heating.

1.3 PBS wash 3 times for 5 min each.

1.4 Dropwise PBS configuration of 0.2% Triton X-100 permeable agent permeable membrane for 10 min. Note, however, that the integrin on the surface of the cell membrane cannot be added with Triton X-100, otherwise it will be washed out.

1.5 Closure: 5% BSA is formulated with PBS and closed at room temperature for 1h.

1.6 PBS dip 3 times for 5 min each.

1.7 (Optional) positive treatment, dilute 10× DNase I Buffer to 1× DNase I Buffer, 100 µl per sample, incubate for 5 min at room temperature; Dilute DNase I with 1 × DNase I Buffer (final concentration of 20 U/ml), aspirate the DNase I Buffer, add 100 µl of DNase I dropwise to each sample, incubate at room temperature for 10 min, PBS wash 3 times for 5 min each.

1.8 Equilibration: Equilibration Buffer for 20 min with 100 µl 1× Equilibration Buffer.

1.9 Labeling: Remove the equilibrium solution, add 50 µl of TdT labeling working solution dropwise per piece (configuration reference struded) to completely cover the sample area, incubate at 37 °C in a wet cassette at 37 °C protected from light for 60 min, and wash three times in PBS for 5 min each.

1.10 Incubate overnight at 4 °C in a humidified chamber of appropriate concentration dropwise.

1.11 PBS dip 3 times for 5 min each.

1.12 Secondary antibody incubation: fluorescent secondary antibody incubated at room

temperature for 1h; PBS dip 3 times to wash off excess secondary antibodies.

1.13 Counterstained nuclei: add 100 µl of 2 µg/ml DAPI counterstained nuclei dropwise and incubate at room temperature for 5min; PBS soak cells 3 times for 5 min each.

1.14 Fluorescence microscope for photography.

2. Frozen section co-staining step

2.1 Freeze slices taken from the refrigerator should be left at room temperature for more than 30 min before immunofluorescence.

2.2 Immerse the slides in 4% paraformaldehyde solution (soluble in 1×PBS) and fix at room temperature for 30 min.

2.3 1× PBS wash 3 times for 3-5 min each time.

2.4 Dropwise PBS configuration of 0.2% Triton X-100 permeable agent permeable membrane 10min. Note, however, that the integrin on the surface of the cell membrane cannot be added with Triton X-100, otherwise it will be washed out.

2.5 Closure: 5% BSA is formulated with PBS and closed at room temperature for 1h.

2.6 PBS dip 3 times for 5 min each.

2.7 (Optional) positive treatment, dilute 10× DNase I Buffer to 1× DNase I Buffer, 100 µl per sample, incubate for 5 min at room temperature; Dilute DNase I with 1 × DNase I Buffer (final concentration of 20 U/ml), aspirate the DNase I Buffer, and add 100 µl of DNase I dropwise to each sample, Incubate at room temperature for 10 min and PBS wash 3 times for 5 min each.

2.8 Equilibration: Equilibration Buffer for 20 min with 100 µl 1× Equilibration Buffer.

2.9 Labeling: Remove the equilibrium solution, add 50 µl of TdT labeling working solution dropwise per piece (configuration reference struded) to completely cover the sample area, incubate at 37 °C in a wet cassette at 37 °C protected from light for 60 min, and wash three times in PBS for 5 min each.

2.10 Incubate overnight at 4 °C in a humidified chamber of appropriate concentration dropwise.

2.11 PBS dip 3 times for 5 min each.

2.12 Secondary antibody incubation: fluorescent secondary antibody incubated at room

temperature for 1h; PBS dip 3 times to wash off excess secondary antibodies.

2.13 Counterstained nuclei: add 100 μl of 2 μg/ml DAPI counterstained nuclei dropwise and incubate at room temperature for 5min; PBS soak cells 3 times for 5 min each.

2.14 Fluorescence microscope for photography.

3. Cell crawling co-staining steps

3.1 Remove the cell crawler from the incubator and wash the cell crawler 1-2 times using PBS.

3.2 In 4% paraformaldehyde solution (dissolved in 1×PBS), leave at 4 °C for 25 min.

3.3 1× PBS wash 3 times for 3-5 min each time.

3.4 Prepared with 1× PBS in a concentration of 0.2% Triton X-100 solution and incubated at room temperature for 5 min for permeable treatment. Note that integrin on the surface of the cell membrane cannot be added with Triton X-100 or it will be washed out.

3.5 Closure: 5% BSA is formulated with PBS and closed at room temperature for 1h.

3.6 Balancing: Equilibration Buffer for 10 min with 100 μl 1×Equilibration Buffer room temperature.

3.7 Labeling: Remove the equilibrium solution, add 50 μl of TdT labeling working solution dropwise per piece (configuration reference above) to completely cover the sample area, incubate at 37 °C in a wet box at 37 °C protected from light for 60 min, wash 2 ml of PBS three times for 5 min each, and aspirate PBS.

3.8 Primary antibody incubation: add 200 μl of antibody dropwise to each crawler (diluted with 5% BSA) and incubate overnight at 4 °C in a wet cassette; PBS soak cells 3 times to wash away the excess antibody for 5 min each time.

3.9 Secondary antibody incubation: fluorescent secondary antibody incubate at room temperature for 1 h; PBS-dip cells wash 3 times to wash off excess secondary antibodies.

3.10 Counterstained nuclei: add 100 μl of 2 μg/ml DAPI counterstained nuclei dropwise and incubate at room temperature for 5 min; PBS soak cells 3 times for 5 min each.

3.11Fluorescence microscope for photography.

Frequently asked questions and suggestions for solutions

- The number of positive cells is small

  Permeability is insufficient, and the permeability step can be optimized by adjusting the incubation time of Protease K or Triton X-100.

- High background (e.g., strong green fluorescent background of unpoptoptotic cells)

  Non-specific incorporation of FITC-12-dUTP. Treatment: Keep cells moist during manipulation; After the labeling reaction is completed, the slides can be washed 3 times with PBS containing 0.1% Triton X-100 and 5 mg/ml BSA for 5 min each after washing once with PBS.

- Tissue slices detach from the slide

  The coating before tissue section adhesion is inadequate. Before unfolding, a microscope slide coated with 3-aminopropyl triethoxysilane (TESPA) works better than poly-L-lysine.

- Microscopy or flow cytometry analyzes only a few cells

  A large number of cells are lost during the procedure. 1. The amount of cells that start can be increased. 2. When preparing the cell suspension, wash the cells with PBS containing 1% BSA during centrifugation.

- Low marking rate

  1. If the sample is fixed with ethanol or methanol, the labeling efficiency is low (cause: chromatin failed to cross-link to the protein at fixation and is lost during the operation), apply 4% paraformaldehyde fixation dissolved in 1×PBS (PH7.4) or formalin or glutaraldehyde fixation.
  2. Excessive fixation leading to excessive crosslinking with proteins can also lead to low labeling efficiency, which can appropriately shorten the fixation time; Or fixed with 2% paraformaldehyde dissolved in 1× PBS.

- False positive

  Some muscle tissue frozen sections such as the production process without paraformaldehyde fixation, prone to endogenous nuclease cutting DNA resulting in false positives, should be

removed from the tissue in time with paraformaldehyde fixed, in addition to try to avoid the use of protease K permeability.

Proteinase K working liquid treatment time, temperature must explore the most suitable conditions, the temperature is too high, the time is too long, easy to destroy the nucleic acid structure, false positive.

- Polylysine-coated slides are prepared:

Pipette 50-100 μl of 0.01% (W/V) aqueous polylysine solution onto the pre-cleaned glass surface. Spread the polylysine solution into thin layers in the area to be used to fix the cells. After the slides are dried, rinse quickly with deionized water, and then let the coated slides stand in the air for 30-60 min until dry. The embedded slides can be stored at room temperature for 3 months.

Note:

This product is for scientific use only.