TUNEL Apoptosis Detection Kit (DAB)

Introduction

The TUNEL Apoptosis Detection Kit (DAB) can be used to detect nuclear DNA fragmentation in tissues or cells during apoptosis. Intracellular DNA endonuclease enzymes are activated during apoptosis, severing genomic DNA between nucleosomes, and DNA is degraded into fragments of 180-200 bp or other integer multiples. The TUNEL kit uses TdT enzyme to incorporate biotin-dUTP onto the 3´-OH ends of fragmented DNA, then uses HRP-labeled streptavidin (Streptavidin-HRP) to bind the biotin-dUTP. Finally, add the DAB substrate to detect apoptosis.

This kit can directly use a common light microscope to detect, which is convenient to use. At the same time, this kit has a wide range of applications, which can be used to detect apoptosis in frozen or paraffin sections, as well as apoptosis in cultured adherent cells or suspension cells.

Components and Storage

Components \ Size	20 rxns	50 rxns	100 rxns	Storage
TdT Enzyme	100 μL	250 μL	500 μL	-20°C
Bition-dUTP	100 μL	250 μL	500 μL	-20°C away from light
5× Reaction buffer	160 μL	400 μL	800 μL	-20°C
TdT Dilution Buffer (optional)	500 μL	1 mL	2 x 1 mL	-20°C
Streptavidin-HRP	22 μL	55 μL	110 μL	-20°C away from light
Streptavidin-HRP Dilution Buffer	1 mL	2 x 1.25 mL	4 x 1.25 mL	-20°C
DAB-A Solution	6 mL	15 mL	30 mL	-20°C away from light
DAB-B Solution	6 mL	15 mL	30 mL	-20°C away from light
Protein K (2 mg/mL)	40 μL	100 μL	200 μL	-20°C
Dnase I	5 μL	10 μL	20 μL	-20°C
10× Dnase I Buffer	100 μL	250 μL	500 μL	-20°C
Shipping: Dry ice	Shelf life: 1 year			

Protocol

1. Materials Not Supplied

1) Reagents: 4% Paraformaldehyde in PBS, PBS, 0.3% H_2O_2 in PBS, 0.3% Triton X-100 in PBS, Xylene (paraffin sections), ethanol (paraffin sections), ddH_2O.

2) Other materials: coverslips/slides, wet box.

2. For paraffin sections

1) Immerse the slides in xylene 2 times, 10 min per time, to completely remove the paraffin.

2) Immerse the slides in absolute ethanol 2 times, 5 min per time. Subsequently, immerse the slides in 90%, 80%, and 70% ethanol for once, 3 minutes each time.

3) Wash the slides 3 times with PBS, 5 min per time. Then carefully blot the excess liquid around the sample on the slide with absorbent paper.

4) Dilute the Proteinase K (2 mg/mL) 1:100 in PBS to make a 20 μg/mL Proteinase K solution. Drop 100 μL Proteinase K (20 μg/mL) on each sample, ensuring the solution can cover the entire sample area. Then incubate at room temperature for 15-30 min.

***Note:** Proteinase K permeabilization time that is too long will increase the risk of tissue sections falling off slides during subsequent wash steps, and too short a time may result in inadequate permeabilization and affect labeling efficiency. For best results, it may be necessary to optimize the incubation time.

5) Wash the slides 3 times with PBS, 5 min per time.

6) Immerse the slides in 0.3% H_2O_2 in PBS and incubate for 20 min at room temperature to block endogenous peroxidase in sections.

***Note:** Do not soak in H_2O_2 solution for too long, as H_2O_2 treatment may cause DNA fragmentation, resulting in false positives.

1) Wash the slides 3 times with PBS, 5 min per time. Gently remove excess liquid and carefully blot the liquid around the sample on the slide with absorbent paper. The treated samples are kept moist in a wet box.

2) Go to step 6.

3. For frozen sections

1) Equilibrate the frozen sections to room temperature. Then immerse the slides in 4% paraformaldehyde solution at room temperature for 30 min.

2) Wash the slides twice with PBS, 5 min per time.

3) Add 100 μL of 0.3% Triton X-100 in PBS to each sample to cover the entire sample area and incubate for 5 min at room temperature.

***Note:** Longer permeabilization time increases the risk of sections falling off the slides during subsequent wash steps, and too short a permeabilization time may result in inadequate permeabilization and affect labeling efficiency. For best results, it may be necessary to optimize the incubation time.

4) Wash the slides 3 times with PBS, 5 min per time.

5) Immerse the slides in 0.3% H_2O_2 in PBS and incubate for 20 min at room temperature to block endogenous peroxidase in sections.

***Note:** Do not soak in H_2O_2 solution for too long, as H_2O_2 treatment may cause DNA fragmentation, resulting in false positives.

6) Wash the slides 3 times with PBS, 5 min per time. Gently remove excess liquid and carefully blot the liquid around the sample on the slide with absorbent paper. The treated samples are kept moist in a wet box.

7) Go to step 6.

4. For adherent cells or cell coverslips

1) Remove the cell culture medium and wash cells once with PBS.

2) Fix cells with 4% paraformaldehyde for 30 min at room temperature.

3) Wash cells twice with PBS to remove residual paraformaldehyde.

4) Add 0.3% Triton X-100 in PBS to each sample to cover the entire sample area and incubate for 5 min at room temperature.

***Note:** Longer permeabilization time increases the risk of sections falling off the slides during subsequent wash steps, and too short a permeabilization time may result in inadequate permeabilization and affect labeling efficiency. For best results, it may be necessary to optimize the incubation time.

5) Wash cells 2 times with PBS.

6) Add 0.3% H_2O_2 in PBS to each sample, ensuring the solution can cover the entire sample area. Incubate for 20 min at room temperature to block endogenous peroxidase in sections.

***Note:** Do not incubate in H_2O_2 solution for too long, as H_2O_2 treatment may cause DNA fragmentation, resulting in false positives.

7) Wash cells 2-3 times with PBS.

8) Go to step 6.

5. For suspension cells or cell suspensions

1) Collect cells and wash cells once with PBS. Then resuspend the cells in PBS at the density of 2 x 10^6 cells/mL.

2) Preparation of cell smears: pipette 50-100 μL of the cell suspension onto a poly-L-lysine coated slide and use a clean slide to gently spread the cell suspension. To make the cells adhere more firmly, dry the sample or use appropriate adhesion reagents.

3) Immerse the smears in 4% paraformaldehyde for 30 min at room temperature.

4) Wash the smears twice with PBS to remove residual paraformaldehyde.

5) Add 100 μL of 0.3% Triton X-100 in PBS to each sample to cover the entire sample area and incubate for 5 min at room temperature.

***Note:** Longer permeabilization time increases the risk of sections falling off the slides during subsequent wash steps, and too short a permeabilization time may result in inadequate permeabilization and affect labeling efficiency. For best results, it may be necessary to optimize the incubation time.

6) Wash the smears twice with PBS.

7) Immerse the smears in 0.3% H_2O_2 in PBS and incubate for 20 min at room temperature to block endogenous peroxidase in sections.

***Note:** Do not soak in H_2O_2 solution for too long, as H_2O_2 treatment may cause DNA fragmentation, resulting in false positives.

8) Wash the smears 2-3 times with PBS.

9) Go to step 6.

6. Positive control treatment (optional):

1) Dilute the 10× DNase I Buffer with ddH_2O to make the 1× DNase I Buffer.

2) For the positive control, add 1× of DNase I Buffer to each sample, making sure the buffer can cover the entire sample area. Then incubated at room temperature for 5 min.

***Note:** For section slides, cell coverslips, and cell smears, add 100 μL 1× of DNase I Buffer to each sample is enough; For plates, the volume of 1× of DNase I Buffer can be adjusted according to the plate size.

3) Dilute the DNase I with 1× DNase I Buffer at the ratio of 1:100 to make the DNase I working solution. Prepare the DNase I working solution freshly every time.

***Note:** DNase I will denature under vigorous mixing, it is recommended not to mix the working solution by vortexing.

1) After incubation, remove the 1× DNase I Buffer. Add the DNase I working solution, making sure the solution can cover the entire sample area. Then incubated at room temperature for 5 min.

***Note:** For section slides, cell coverslips, and cell smears, add 100 μL DNase I working solution to each sample is enough; For plates, the volume of DNase I working solution can be adjusted according to the plate size.

2) After incubation, remove the DNase I working solution and wash 2-3 times with PBS.

7. Preparation of biotin labeling solution:

1) Dilute the 5× Reaction buffer with ddH_2O to make 1× Reaction buffer.

2) Refer to the table below to prepare the biotin labeling solution, and the labeling solution needs to be prepared freshly every time.

	Experimental group/positive control	Negative control (optional)
TdT Enzyme	5 μL	0 μL
Biotin-dUTP	5 μL	5 μL
1× Reaction buffer	40 μL	45 μL

Total	50 μL	50 μL

***Note:** The 50 μL labeling system is suitable for section slides, cell coverslips, and cell smears. For plates, the volume of labeling solution can be adjusted according to the size of the plate to make the labeling solution fully cover the cells.

8. Sample biotin labeling

1) Add 50 μL of biotin labeling solution to each sample and incubate at 37°C for 1 h in the dark.

***Note:** It is recommended to cut parafilm to cover the samples to ensure that labeling solution evenly covers the cell surface. If plates are used, PBS can be added to the plate interstitial or excess wells to reduce evaporation.

2) After incubation, remove the biotin labeling solution and wash cells twice with PBS.

9. Streptavidin-HRP labeling and DAB colorimetric detection:

1) Refer to the table below to prepare the Streptavidin-HRP working solution. The Streptavidin-HRP Dilution Buffer is viscous, so it is recommended to prepare one more well.

	1 sample	5 samples
Streptavidin-HRP	1 μL	5 μL
Streptavidin-HRP Dilution Buffer	49 μL	245 μL
Total	50 μL	250 μL

***Note:** The 50 μL labeling system is suitable for section slides, cell coverslips, and cell smears. For plates, the volume of labeling solution can be adjusted according to the size of the plate to make the labeling solution fully cover the cells.

2) Add 50 μL Streptavidin-HRP working solution to each sample, then incubate at 37°C away from light for 30 min.

***Note:** It is recommended to cut parafilm to cover the samples to ensure that labeling solution evenly covers the cell surface. If plates are used, PBS can be added to the plate interstitial or excess wells to reduce evaporation.

3) Prepare the DAB working solution: Mix DAB-A Solution and DAB-B Solution at a ratio of 1:1 to make the DAB working solution. 0.2-0.5 mL of DAB working solution is needed for each sample. The DAB working solution needs to be prepared immediately before use, and is advisable to be used within 30 min after preparation.

4) After incubation, remove the Streptavidin-HRP working solution and wash cells 3 times with PBS.

5) Add 0.2-0.5 mL of DAB working solution to each sample and incubate for 5-30 min at room temperature in the dark. If the reaction is too strong and the color is too deep, the incubation time can be less than 5 min, if the reaction is very weak, the incubation time can be appropriately extended, or even overnight.

6) Remove the DAB working solution and wash the cells with PBS, it is recommended to wash 3-4 times.

7) (Optional) Stain the nuclei with hematoxylin staining solution or methyl green staining solution. Subsequent wash cells 3 times with PBS.

8) Observe the results directly under the light microscope. Slides or smears also can be observed after mounting. Dehydration with 95% ethanol for 5 min, dehydration with 100% ethanol twice, 3 min each time,

clear with xylene twice, 5 min each time, then observation after mounting.

Troubleshooting

Observation	Possible cause	Recommendation
Non-specific staining	TdT enzyme concentration is too high	Reduce the concentration of TdT enzyme; dilute the TdT enzyme 1:2-1:10 using the TdT Dilution Buffer provided in the kit
	Excessive TdT enzyme reaction time or the samples dry during labeling	Optimize the reaction time and avoid the sample drying out
	High endogenous nuclease activity causes DNA breaks during fixation	Ensure the sample is fixed immediately after sampling
	Inappropriate choice of fixative	Use the recommended fixative
	Insufficient washing	Increase the number of washes and the duration of each wash
Low labeling	Chromatin and protein are not cross-linked due to fixation with ethanol or methanol	Use 4% paraformaldehyde for fixation or use formaldehyde or glutaraldehyde
	Over-fixation	Reduce fixation time
	Incomplete deparaffinization of paraffin sections	Extend deparaffinization time or change a fresh deparaffinization solution
	Permeabilization is not enough	Increase permeabilization time or optimize the concentration and duration of the permeabilizing agent
High background staining	Mycoplasma contamination	Use a mycoplasma staining test kit to check for contamination
	The concentration of TdT enzyme is too high or the reaction time is too long	Lower the concentration of TdT enzyme or optimize the reaction time
	Incomplete blocking with hydrogen peroxide	Improve the peroxidase blocking method, such as extending the blocking time
	DAB incubation time is too long	Reduce DAB incubation time
Sample detachment	Overly long permeabilization time	Optimize the permeabilizing agent's processing time
	Inadequate coating before adhesion	Use 3-aminopropyltriethoxysilane (TESPA) for coating microscope slides, which is more effective than poly-L-lysine

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