Product Information

Biotin TUNEL Assay Apoptosis Detection Kit

Catalog Number: T6068

Product Size: 20 T, 50 T

Contents:

Component	20 T	50 T
A. Biotin TUNEL Reaction Buffer	2×0.5 mL	5×0.5 mL
B. TdT enzyme	20 μL	50 μL
C. Streptavidin-HRP	20 μL	50 μL
D. Streptavidin-HRP Dilution	1 mL	2×1.25mL
E. DAB solution-A	100 μL	250 μL
F. DAB solution-B	1 mL	2.5 mL
G. DAB solution-C	50 μL	125 μL
H. Proteinase K (2 mg/mL)	40 μL	100 μL
I. DNase I (2 U/μL)	5 μL	13 μL
J. 10 × DNase I Buffer	100 μL	260 μL

Storage

This product should be stored at -20 ℃ ; TUNEL Reaction Buffer should be stored in dark at -20 ℃ and avoid repeated freezing and thawing. When stored as directed, product is stable for at least 6 months.

Note: the toxic and carcinogenic ingredients such as solid cacodylate trihydrate and cobalt chloride are contained in the DAB solution and TUNEL Reaction Buffer. Please wear masks and gloves when using. If contacting the skin, please wash with plenty of water immediately. Please treat the waste liquid as toxic substance.

Description

A remarkable feature of apoptosis is the degradation of chromosome DNA, which is a common phenomenon. The degradation is very specific and regular. DNA fragments break into different lengths are of 180 bp-200 bp, showing a specific ladder like ladder pattern in agarose gel electrophoresis. The kit uses TUNEL method and uses Terminal Deoxynucleotidyl Transferase(TdT) catalyzes the incorporation of Biotin-dUTP into the 3´-OH terminal of the broken DNA of apoptotic cells. Then it was specifically combined with Streptavidin labeled with horseradish peroxidase (Streptavidin-HRP). Finally, under the catalysis of HRP, the apoptotic cells were displayed by DAB, so that the apoptotic cells could be observed and counted by ordinary optical microscope. TUNEL can selectively detect apoptotic cells rather than necrotic cells or cells with DNA strand breaks caused by irradiation and drug therapy. In this experiment, TdT enzyme catalyzes the incorporation of dUTP into the 3'-OH end of the broken DNA strand.

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Protocol

Experimental materials (self provided)

PBS, pH=7.4

4% paraformaldehyde (in PBS)

0.3% H_2O_2 in PBS (Fresh configuration)

Solvent for dewaxing (for paraffin section sample)

1. Sample preparation

1.1 Cell sample

1). Optional: prepare a negative control sample (add TUNEL reaction mixture without TdT enzyme).

2). Wash cells twice with PBS.

3). Cell fixation: add appropriate 4% paraformaldehyde, and place at 4 ℃ for 30 min.

4). Wash cells twice with PBS.

5). Cell permeabilization: cells can be permeated with 0.2% Triton X-100 in PBS and placed at room temperature for 20 min.

6). Wash cells twice with PBS.

7). Block cells: add about 100 μL 0.3% H2O2 solution in PBS into each well, tap the well plate to make it fully cover the cells, block cells at room temperature in dark for 30 min, and wash cells twice with 1 × PBS.

1.2 Paraffin section

1). Paraffin sections were immersed in dimethylbenzene twice at room temperature for 5 min each time to remove paraffin thoroughly.

Note: dimethylbenzene is toxic and volatile. Please operate in the fume hood.

2). At room temperature, the slices were immersed in absolute ethanol and rinsed twice for 5 min each time.

3). At room temperature, the section samples were immersed in ethanol (95%, 90%, 80%, 70%) of different concentration gradients, and each concentration was rinsed once for 5 minutes.

4). Immerse the slice in pure water for 3 minutes at room temperature, and then immerse the slice in 1 × PBS for 3 minutes. Carefully suck the excess liquid around the slice sample with filter paper.

5). The contour of the sample was drawn around the section sample with an immunohistochemical pen so that it could be penetrated and labeled downstream.

6). Dilute 2 mg/mL of proteinase K solution with 1 × PBS in a ratio of 1:100, so that its final concentration is 20 μg/mL. Add 100 μL diluted proteinase K solution to each sample, make the solution cover all sample areas, and incubate at room temperature for 20 min. The incubation time and temperature of proteinase K need to be optimized according to different types of tissue samples.

Note: protease K can help to penetrate tissues, but prolonging incubation time may cause slices to fall off and too short may cause penetration incomplete, so it is necessary to optimize incubation time. Generally, the time is 10-30 min. Section of 4 μm may need 10 min, section of 30 μm should be extend to 30 min.

7). Wash cells twice with PBS.

8). Block cells: add about 100 μL 0.3% H2O2 solution in PBS into each well, tap the well plate to make it fully cover the cells, block cells at room temperature in dark for 30 min.

9). Infiltrates and cleans the slices twice with PBS for 5 min each time. The excess liquid is sucked by the filter paper, and place processed sample in wet box to keep wet.

Note: in this step, protease K must be washed clean thoroughly, otherwise it will seriously interfere with the subsequent labeling reaction.

1.3 Frozen tissue section

1). Place frozen slices on a shelf at room temperature and dried for 20 minutes.

2). The slides were immersed in 4% polyformaldehyde (in PBS) and fixed at room temperature for 30 min.

3). PBS infiltrates and cleans slices with PBS twice for 5 min

each time.

4). Carefully suck the liquid around the sample on the slide with filter paper.

5). Dilute 2 mg/mL of proteinase K solution with 1 × PBS in a ratio of 1:100, so that its final concentration is 20 μg/mL. Add 100 μL diluted proteinase K solution to each sample, make the solution cover all sample areas, and incubate at room temperature for 20 min. The incubation time and temperature of proteinase K need to be optimized according to different types of tissue samples.

Note: protease K can help to penetrate tissues, but prolonging incubation time may cause slices to fall off and too short may cause penetration incomplete, so it is necessary to optimize incubation time. Generally, the time is 10-30 min. Section of 4 μm may need 10 min, section of 30 μm should be extend to 30 min.

6). Wash cells twice with PBS.

7). Block cells: add about 100 μL 0.3% H2O2 solution in PBS into each well, tap the well plate to make it fully cover the cells, block cells at room temperature in dark for 30 min.

8). Infiltrates and cleans the slices twice with PBS for 5 min each time. The excess liquid is sucked by the filter paper, and place processed sample in wet box to keep wet.

1.4 positive treatment (this step is only for positive control, and other samples are subject to TUNEL reaction directly)

1). Dilute 10 × DNase I buffer with ddH_2O in the proportion of 1:10 to 1 × DNase I buffer for standby.

2). Add 100 μL 1 × DNase I buffer to the transparent sample, and incubate at room temperature for 5 min.

3). Dilute DNase I (2 U/μL) with 1 × DNase I buffer in the proportion of 1:100 to a final concentration of 20 U/mL.

4). Gently suck off the excess liquid, add 100 μL DNase I working solution(20 U/mL) , and incubate at room temperature for 10 minutes.

5). Gently suck off the excess liquid and clean the sample twice with PBS.

2. TUNEL Reaction

2.1 Prepare TUNEL reaction mixture in advance: added with 1 μL TdT enzyme into 49 μL Biotin-TUNEL Reaction Buffer. Each sample needs 50 μL Biotin-TUNEL reaction mixture.

2.2 Add 50 μL TUNEL Reaction Solution to each sample , incubated at 37 ℃ in dark for 60 min, and tissue samples needed 2 h (negative control sample was added with TUNEL Reaction Solution without TDT enzyme).

Note: 50 μL TUNEL Reaction Solution is suitable for smear, slice or one well of 96 well-plate, 48 well-plate, 24 well-plate or 12 well-plate. If it is one well of 6 well-plate, 100 μL TUNEL Reaction Solution is recommended. If the sample to be tested is smear, slice or in 24 well-plate, 12 well-plate or 6 well-plate, it is recommended to cover the sample with anti evaporation film after adding TUNEL Reaction Solution, so as to prevent the evaporation of TUNEL Reaction Solution, and make TUNEL Reaction Solution cover the sample evenly.

2.3 Wash cells twice with PBS.

3. Preparation of Streptavidin-HRP working solution and DAB mixture solution

3.1 Preparation of Streptavidin-HRP working solution (Fresh configuration)

	For one sample
Streptavidin-HRP	1 μL
Streptavidin-HRP Dilution	49 μL
Total volume of Streptavidin-HRP working solution	50 μL

3.2 Preparation of DAB mixture solution (Fresh configuration)

	For one sample
DAB solution-A	5 μL
DAB solution-B	42.5 μL
DAB solution-C	2.5 μL
Total volume of DAB mixture solution	50 μL

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4. Sample coloration

4.1 Add 50 μL Streptavidin-HRP working solution to the sample, and incubate at 37 ℃ in dark for 30min.

Note: 50 μL Streptavidin-HRP working solution is suitable for smear, slice or one well of 96 well-plate, 48 well-plate, 24 well-plate or 12 well-plate. If it is one well of 6 well-plate, 100 μL Streptavidin-HRP working solution is recommended. If the sample to be tested is smear, slice or in 24 well-plate, 12 well-plate or 6 well-plate, it is recommended to cover the sample with anti evaporation film after adding Streptavidin-HRP working solution, so as to prevent the evaporation of Streptavidin-HRP working solution, and make Streptavidin-HRP working solution cover the sample evenly.

4.2 Wash cells twice with PBS.

4.3 Add 50 μL DAB mixture solution to the sample, incubate at room temperature for 5-30 min or master the dyeing time according to the development of color under the microscope.

Note: if the color rendering is very strong, it can be stopped within 5min. If the color rendering is very weak, the color rendering time can be extended appropriately, or even overnight.

4.4 Wash cells twice with PBS.

4.5 Optional: stain nuclear with hematoxylin staining solution or methyl green staining solution. Then wash with PBS for 3 times.

4.6 Observe and take photos under optical microscope, or dehydrate with 95% ethanol for 5 min, then dehydrate with 100% ethanol for 2 times, about 3 min each time, then transparent with xylene for 2 times, 5 min each time, then observe by sealing, and take photos under optical microscope.

Notes

1. For your safety and health, please wear lab coats and disposable gloves.

2. Sodium azide can inhibit HRP. Do not use reagents containing sodium azide in the experiment.

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