

CoraLite[®] 488-594 TUNEL Apoptosis

Instructions

Materials required but not supplied with kit:

- 1. PBS buffer (pH~7.4)
- 2. 4% paraformaldehyde (in PBS)
- 3. Bovine serum albumin (BSA) or normal sheep/bovine serum
- 4. 70% ethanol (optional)
- 5. Dewaxing solvent (if using paraffin section samples)

Cell samples

- 1. Prepare a negative control sample (add TUNEL reaction solution without TdT enzyme).
- 2. Wash the cells twice with PBS.
- 3. Add 4% paraformaldehyde (pH 7.4) solution in excess and incubate at 4°C for 30 min.
- 4. Wash the cells twice with PBS.
- 5. Add 70% ethanol pre-cooled on ice and incubate at -20°C for 4 h. Cells can be stored for one week in 70% ethanol at -20°C. Alternatively, the cells can be permeabilized with 0.2% Triton X-100 in PBS solution and incubated at room temperature for 20 minutes.
- 6. Wash the cells twice with PBS.



Paraffin tissue sections

- Immerse slides in xylene for 5 minutes. Repeat this step in fresh xylene for a further 5 minutes.
 Note: Xylene is toxic and volatile. Please perform this in a fume hood.
- 2. Immerse slides in absolute ethanol for 5 minutes. Repeat this step for a further 5 minutes in fresh ethanol.
- 3. Rehydrate sections by sequentially incubating with 100%, 95%, 80%, and 60% ethanol for 5 minutes each.
- 4. Immerse slides in distilled water for 3 minutes each. Use filter paper to carefully absorb the excess liquid around the sample.
- 5. Use an immunohistochemical pen to trace around the samples.

6. Dilute Proteinase K solution 1:100 in 1mL of PBS to a final concentration of 20 μ g/mL. Add 20 μ g/mL Proteinase K solution to each sample in a dropwise manner until the solution covers the entire area of the sample (~100 μ L), followed by incubation for 20min at room temperature. (The incubation time and temperature of Proteinase K should be optimized according to your tissue samples.)

Note: Over-incubation with Proteinase K may cause the sections to fall off, so optimize the length of the incubation time. Typically, the time required is 10-30 minutes. For a section of around 4 µm: 10 minutes, and for sections around 30 µm: 30 minutes.

7. Wash the sections with PBS twice for 5 minutes each time, absorb the excess liquid with filter paper, and place the processed sample in a humid box to keep the sample moist.

Note: Proteinase K must be thoroughly washed off during this step to prevent interference with subsequent labeling.



Frozen tissue sections

- 1. Place the frozen sections on a rack at room temperature for 20 minutes to thaw.
- 2. Immerse the slides in 4% paraformaldehyde solution (in PBS) and fix at room temperature for 30 min.
- 3. Wash the slides twice with PBS for 5 minutes each time.
- 4. Use filter paper to carefully dry the liquid around the sample on the slide.
- 5. Dilute Proteinase K solution 1:100 in 1mL of PBS to a final concentration of 20 μ g/mL. Add 20 μ g/mL Proteinase K solution to each sample in a dropwise manner until the solution covers the entire area of the sample (~100 μ L), followed by incubation for 20min at room temperature.

Note: Over-incubation with Proteinase K may cause the sections to fall off, so optimize the length of the incubation time. Typically, the time required is 10-30 minutes. For a section of around 4 μ m: 10 minutes, and for sections around 30 μ m: 30 minutes.

6. Wash the sections with PBS twice for 5 minutes each time, absorb the excess liquid with filter paper, and place the processed sample in a humid box to keep sample moist.

Note: Proteinase K must be thoroughly washed off during this step to prevent interference with subsequent labeling.

Positive control treatment (only your positive control sample needs to perform this step, your main experimental samples directly undergo the TUNEL reaction in step 5)

- 1. Dilute the 10 X DNase I Buffer with ddH2O in a ratio of 1:10 to make 1 X DNase I Buffer as a working concentration.
- 2. Add 100 μL 1 X DNase I Buffer dropwise to your sample, and incubate at room temperature for 5 minutes.
- 3. Dilute DNase I (2 U/ μ L) 1:100 with 1 X DNase I Buffer for working solution with a final concentration of 20 U/mL.
- 4. Gently aspirate the excess liquid. Next add 100 μ L of 20 U/mL DNase I working solution to your slides in a dropwise manner, and incubate at room temperature for 10 min.
- 5. Gently aspirate the excess liquid, and wash the sample twice with PBS.



TUNEL reaction

1. Prepare a master mix of the TUNEL reaction mixture in advance: for each sample allow 50 µL TUNEL reaction buffer with 1 µL TdT enzyme.

2. Add 50 µL of the TUNEL reaction mixture to each sample.

- i. For adherent cells, evenly cover the sample with the buffer solution before carefully placing a glass coverslip. Incubate the samples in the dark at 37°C for 60 min.
- ii. Suspended cells can be added to a microtiter plate and incubated on a plate shaker, alternatively you can gently shake the reaction tube every 15 minutes. Incubate in the dark at 37°C for 60 min.
- iii. For tissue samples, cover the sample evenly with buffer and carefully place a glass cover slip. Place the sample in a humid box and incubate at 37°C for 2 hours. Spread a paper towel with a small amount of water on the bottom of the wet box to maintain humidity. Incubate for 2 h at 37°C in the dark.
- 3. Gently aspirate the reaction solution. Next, soak and rinse twice for 5 mins in a 1X PBS staining tank. Then use an appropriate amount of 0.1% Triton X-100 in PBS containing 5 mg/mL BSA buffer to wash the sample 3 times, 5 min each time to reduce background.

4. Counterstaining (optional): Add 2 µg/mL DAPI dropwise to each sample and incubate for 10 min at room temperature in the dark. Next, remove stain and rinse the slides 3 times in 1X PBS for 5 minutes each time.

5. Mounting (optional):

- i. Immerse the slides in distilled water for 5 minutes.
- ii. Dehydrate sections by sequentially incubating with 100%, 95%, 80%, and 60% ethanol for 5 minutes each.
- iii. Then immerse slides in fresh xylene for 5 minutes and repeat this step for another 5 minutes in fresh xylene. (Note: xylene fumes are toxic so perform this step in the hood).
- iv. Carefully wipe off the liquid around the sample and dropwise add 50 μ L antifade mounting solution to the samples. Add a coverslip to the samples using the blunt ends of tweezers and gently tap the coverslip to remove air bubbles.
- 6. Observe and analyze with a fluorescence microscope. CL488 is a green fluorescent dye with an excitation wavelength/emission wavelengths of 490/515 nm, respectively. CL594 is a red fluorescent dye with an excitation/emission wavelengths of 590/617 nm. Apoptotic cells are marked with bright green fluorescence. The negative control sample (those without TdT) should not have detectable signal.