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.

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Paraffin tissue sections

1. **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 µg/mL. Add 20 µg/mL Proteinase K solution to each sample in a dropwise manner until the solution covers the entire area of the sample (~100 µL), 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 μg/mL. Add 20 μg/mL Proteinase K solution to each sample in a dropwise manner until the solution covers the entire area of the sample (~100 μL), 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 μm: 10 minutes, and for sections around 30 μm: 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/μL) 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 μL 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.**

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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 100µL of the equilibration buffer to each sample and incubate for 5 minutes.**

3. **Discard the equilibration buffer, carefully aspirate the excess liquid around the sliced samples with filter paper, and 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.*

4. **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.**

5. **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.**

6. **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 µL 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.*

7. **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.**