TransDetect® In Situ Fluorescein TUNEL Cell Apoptosis Detection Kit

Cat. No. FA201

Storage: TdT at -20°C for one year, 1×Labeling Solution at -20°C in dark for one year

Description

TransDetect® In Situ Fluorescein TUNEL Cell Apoptosis Detection Kit provides a precise, simple and low-toxicity way to detect and quantify apoptotic cell death at single cell level in cells and tissues. TdT-mediated dUTP Nick-End Labeling (TUNEL) reaction preferentially labels DNA strand breaks generated during apoptosis with fluorescein-labeled dUTP. The fluorescein labeled DNA can be detected and quantified by fluorescence microscopy or flow cytometry. This kit can be used to detect apoptosis in paraffin-embedded tissue sections, cryopreserved tissue sections, cells cultured on chamber slides, cell smear and cell suspensions.

Highlights

- Extremely low toxicity, labeling solution is free of commonly used highly-toxic cacodylate.
- High sensitivity, high specificity, optimal ratio of labeled and non-labeled substrates.
- Simple, one-step labeling by mixing TdT and labeling solution.
- Flexible, can be used in many assay systems.

Kit Contents

Component	FA201-01 (25 rxns)	FA201-02 (50 rxns)
TdT	50 µl	100 µl
1×Labeling Solution	1.25 ml	2×1.25 ml

Additional Reagents Required

- Formaldehyde fixing solution（3% formaldehyde and 2% sucrose in PBS）
- Permeabilisation solution（0.1% Triton X-100 in PBS）
- DNase I (Cat. No. GD201), 10×DNase I Reaction Buffer
- Anti-Fade solution

Procedure

A. Paraffin-embedded tissue

1. Dewax and rehydrate

   Dewax by washing in xylene and rehydrate through a series of gradient concentration of ethanol (eg. 95%, 90%, 85%, 75%, 50%) and double distilled water. Then rinse the slides with PBS and carefully remove liquid around the section. Make sure to keep the surface of slides moisturized.

2. Permeabilisation

   Apply 100 µl of permeabilisation solution to the slides; incubate at room temperature for 5 minutes.

3. Labeling

   Mix 50 µl of 1×Labeling Solution and 2 µl of TdT thoroughly. Then apply to the slide surface. Incubate at 37°C in the dark for 1 hour.

   （note: volume of 1×Labeling Solution can be adjusted according to the sample size. Labeling solution should cover the whole sample; insufficient liquid could result in failure of labeling. Use a humidified chamber to keep the slide moisturized. Starting from this step, avoid exposing to light to prevent fluorescence quenching.）

4. Rinse with permeabilisation solution for 3 times, 5 minutes each time.
5. Carefully remove liquid around the section. Add appropriate volume of Anti-Fade solution. Samples is ready to be analyzed by flow cytometry or fluorescent microscope.

B. Cyropreserved tissue

1. Fixation
   For unfixed sections, use formaldehyde fixing solution to fix at room temperature for 30 minutes. Rinse with PBS for 3 times, 5 minutes each time. For fixed section, proceed to step 2.
2. Same as steps 2-5 in paraffin-embedded tissue section.

C. Fixed cell slides

1. Prepare adherent cells on slides or cell smear.
2. Fixation
   Use formaldehyde fixing solution to fix at room temperature for 30 minutes. Rinse with PBS for 3 times, 5 minutes each time.
3. Permeabilisation
   Add enough permeabilisation solution to immerse the slides. Incubate at room temperature for 5 minutes.
4. Same as steps 3-5 in paraffin-embedded tissue section.

D. Cell suspensions

1. Centrifuge the suspension at 500×g for 5 minutes. Discard the supernatant, rinse the pellet with PBS twice. Centrifuge at 500×g for 5 minutes. (suggested cell number≥1×10^5 , but too many cell number should also be avoided)
2. Fixation
   Add enough formaldehyde fixing solution, carefully resuspend cells by inversion. Incubate at room temperature for 30 minutes. Resuspend by inverting 2-3 times during incubation.
3. Rinse with PBS for 3 times, centrifuge at 500×g for 5 minutes. Discard the supernatant.
   (note: rinse gently invert and resuspend before centrifugation.)
4. Permeabilisation
   Add enough permeabilisation solution, gently resuspend cells and incubate at room temperature for 10 minutes, resuspend by inverting 2-3 times during incubation.
5. Labeling
   Centrifuge at 500×g for 5 minutes, discard the supernatant. Mix 50 µl of 1×Labeling Solution and 2 µl of TdT thoroughly at the ratio of 25:1 and then add the mixture into the cells. Incubate at 37°C in the dark for one hour (resuspend by inverting several times during incubation).
6. Centrifuge at 500×g for 5 minutes, discard the supernatant. Rinse the pellet with permeabilisation solution twice and resuspend it with PBS. Detect by flow cytometry or fluorescent microscope.

E. Positive control

1. Treat the positive control for permeabilisation as described above. Dilute 10×DNase I Reaction Buffer with permeabilisation solution to 1× and then add DNase I (final concentration at 10-15 U/ml). Mix thoroughly and add to the positive control. Incubate at room temperature for 15-30 minutes.
2. Rinse positive control with permeabilisation solution for 3 times, 5 minutes each time.
3. Labeling and following steps are the same as described steps in section A-D.

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