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Technical Information

Flamma® Fluors TUNEL Assay Kit

Overview

Apoptosis is a highly regulated and controlled process that confers advantages during an organism's lifecycle, and defective apoptotic processes have been implicated in a variety of diseases such as atrophy and cancers. In situ labeling of apoptotic cells allows highly sensitive and quantitative analysis of involved cells. Characteristics of the late stages of apoptosis are changes in nuclear morphology, including chromatin condensation, degradation of nuclear envelope, and DNA strand breaks. TUNEL assays are the most widely used methods for rapid identification and quantification of the apoptotic cell fraction in cultured cell preparations. TUNEL reaction preferentially labels DNA strands that generated during apoptosis using the ability of TdT to label blunt ends of double-stranded DNA independent of a template. This selective labeling allows discrimination of apoptosis from necrosis and from primary DNA strand breaks induced by cytostatic drugs or irradiation. BioActs developed Flamma® Fluors TUNEL assay kit for quantitative analysis of apoptotic cells in the mixed cell populations. The kit employs dUTP conjugated Flamma® Fluors and FITC dyes for labeling of fixed cells or tissues. Flamma® Fluor dyes display excellent fluorescence activity and photostability after conjugated to biomolecules, allowing detection of low-abundance biological structures with great sensitivity. Flamma® TUNEL assay kit is reliable and effective method to detect and quantify a wide levels of apoptotic cells by fluorescence microscopy and flow cytometry. In addition, the kit can be used in the analysis of apoptotic cell of paraffin-embedded tissue sections along with cultured cells. BioActs offers Flamma® TUNEL assay kit for the detection apoptotic cells in frozen and formalin-fixed tissue sections, determination of the sensitivity of malignant cells to drug-induced apoptosis, discrimination of apoptotic and necrotic cells in the cell death environment, etc.

Table 1. Components of Flamma® Fluors TUNEL assay kit

Components	Volume for 1 test	Total Volume (50 tests)
dUTP conjugated dye	5 µL	250 µL
5 x reaction buffer	10 µL	1,500 µL
TdT enzyme, recombinant	1 µL	50 µL
Deionized water	35 µL	1.75 mL

Table 2. List of Flamma® Fluors TUNEL assay kits

Cat. No.	Product	λ_{Ex}*	λ_{Em}*	Packing unit
XTN1101	FITC TUNEL assay kits	495	522	50 tests
XTN1201	Flamma® 552 TUNEL assay kits	551	570	50 tests
XTN1301	Flamma® 648 TUNEL assay kits	648	672	50 tests

*maxima of excitation and emission in nm

• Required materials for experiment

- 1x PBS
- Propidium iodide
- 1.5 mL micro centrifuge tube
- 50 mL conical tube,
- Micropipettes
- Poly-L-lysine coating or salinized chamber slides
- Glass cover slides
- Centrifuge
- Humidified chamber
- Incubator (37 ℃)
- Flow cytometry
- Fluorescence microscope
- Rubber cement or transparent manicure

• Required materials for suspended cells

- 1% paraformaldehyde in PBS buffer
  *Paraformaldehyde can be replaced with methanol-free formaldehyde
- 0.2% Triton® X-100 solution in PBS buffer
  *0.2% Triton® X-100 solution can be replaced by 70% ethanol
- 20 mM EDTA (pH 8.0)
- 0.1% Triton® X-100 solution in PBS containing 5 mg/mL BSA
- DNase-free RNase A
- Camptothecin (CPT) dissolved in DMSO

• Required materials for fixed cells

- 4% paraformaldehyde in PBS (pH 7.4)
- 0.2% Triton® X-100 solution in PBS
  *0.2% Triton® X-100 solution can be replaced by 70% ethanol
- DNase-free RNase A
  - (Optional) 70% ethanol
  - (Optional) DAPI (Vector Labs Cat. # H-1500)
  - When setting positive control, DNase I
  - When setting positive control, DNase I buffer

• Required materials for paraffin-embedded tissue sections

- 4% paraformaldehyde / PBS (pH 7.4)
  *Paraformaldehyde can be replaced with methanol-free formaldehyde
- Xylene
- 100, 95, 85, 70 and 50% ethanol
- 0.85% aqueous NaCl solution
- Proteinase K in PBS
- Proteinase K Buffer
  - When setting positive control, DNase I
  - When setting positive control, DNase I buffer

Experimental protocol

If positive control is required, prepare the following before conducting the experiment.

Positive control

① Prepare cultured cells of 2 x 10^6 cells.

② Divide the samples into two groups. One group is treated with 2 μg/mL of CPT in DMSO to of 0.2 μg/mL, and the other group is treated with DMSO in the same amount as CPT.

ex. Group 1: Add 1 mL of 2 μg/mL CPT in DMSO to 10 mL of medium
Group 2: 10 mL of medium was treated with 1 mL of DMSO

③ The two groups of samples are incubated at 37 ° C for 5 h.

④ After collecting the cells and media, prepare samples for the following experiment and analyze with flow cytometry.
Note: Cells stained with the Flamma Fluors TUNEL assay kit and PI-stained cells, respectively, are prepared with the sample as a set-up control of flow cytometry.

• Suspended cells

1. Cleaning: cells are washed twice with PBS, centrifuged (300 x g) at 4 ° C, suspend again in 0.5 ml PBS.
2. Fixing: 5 mL of 1% paraformaldehyde in PBS (pH 7.4) is injected to cells and the cells fixed for 20 min at ice-cold condition.
3. Cleaning: Centrifuge (300 × g) at 4 ° C, suspend the cells in 5 mL of PBS. Repeat centrifuge and suspend the cells in 0.5 mL PBS.
4. Permeabilization: Add 5 mL of 0.2% Triton® X-100 solution in PBS and incubate at 25 ℃ for 5 min.
   Remark: 0.2% Triton® X-100 solution in PBS can be replaced with 70% ice-cold ethanol.
   In this case, stand sample at -20 ℃ for 4 hours.
   Centrifuge sample for 10 min (300 x g), add 5 mL of PBS and suspend.
   Repeat centrifuge and suspend the cells in 1 mL PBS.
5. Buffer Equilibration: Transfer the suspended cells to a 1.5mL microcentrifuge tube, centrifuge for 10 min (300 × g), suspend in 100 μL of 5 × reaction buffer diluted to 1 ×, and incubate at 25 ℃ for 5 min.
6. Staining solution preparation: prepare the staining reagent during the process.
   Prepare a sufficient amount of dye reagent, including positive control, refer below table.
   Note:
   - *1 assay is based on 5 cm^2 of sample area. If the area is larger than that, the amount of reagents should be increased in proportional to the area.*
   - *Make sure that the dyeing reagent is on ice and not exposed to light*
   - *If negative control settings are required, set the sterilized distilled water to 36 μL, excluding the rTdT enzyme.*
7. Staining: Centrifuge (300 × g) for 10 min, add 50 μL of staining reagent, suspend the cells, and stand at 37 ℃ for 1 h.
   Remark: suspend the cells every 15 minutes with a microwave pet.
   avoid the microcentrifuge tube from light.
8. Quenching staining: 1 mL of 20 mM EDTA is added to reaction the, and gently shake the mixture.
9. Cleaning: Centrifuge (300 × g) for 10 min and suspend the cells in 1 mL of 0.1% Triton® X-100 solution in PBS containing 5 mg/mL BSA. Repeat the step.
10. Nuclear staining (PI staining): Centrifuge (300 × g) for 10 min and suspend the cells in 0.5 mL of DNase-free RNase A and fresh 250 μg of propidium iodide solution (5 μg/mL in PBS).
11. Stain the cells at ℃ for 30 minutes in a dark.
12. Analysis: Cells are analyzed at two wavelengths using flow cytometry.
    Remark: Cells stained with Flamma Fluors TUNEL assay kit and cells stained with PI are each setup control of flow cytometry.

• Fixed cells

1. Attach cells to slides: Cells are incubated in 70-80% of the Lab-Tek® chamber slides and then washed twice with PBS at 4 ℃.
2. Fixing: Place slides into a 50 mL conical tube containing 4% paraformaldehyde in PBS (pH 7.4) and fix at 4 ℃ for 25 min.
3. Cleaning: The slides are placed in a 50-mL conical tube containing PBS solution at 25 ℃, soaked for 5 minutes, repeat PBS soaking.
   NOTE: cleaned slides can be stored in PBS at -20 ° C or in 70% ethanol at 4 ℃ for up to 2 weeks.
4. Permeabilization: Immerse the slides in a 50 mL conical tube containing 0.2% Triton® X-100 solution in PBS at 25 ℃for 5 min.
5. Cleaning: The slides are placed in a 50-mL conical tube containing PBS solution at 25 ℃, washed by immersion for 5 min,

repeat PBS soaking.

If positive control is required, prepare at this stage

• Positive control

① Cover the fixed cells with 100 μL of DNase I buffer, and incubate at 25 ℃ for 5 minutes.

② Remove the DNase I buffer. Cover slide with 100 μL of DNase I buffer containing DNase I A (5.5 ~ 10 unit/mL) and incubate at 25 ℃ for 10 min.

③ Shake the slides gently to discard the solution, and place slides into a 50 mL conical tube containing distilled water and wash 3 or 4 times.

6. Buffer Equilibration: Remove the PBS on the slide by tapping the slides, cover the cells with 100 μL of 5X reaction buffer diluted to 1X, and incubate at 25 ℃ for 5-10 min.
7. Staining solution preparation: Prepare sufficient amount of staining reagent, including positive control, refer below table.

Note:

- *1 assay is based on 5 cm^2 of sample area. If the area is larger than that, the amount of reagents should be increased in proportional to the area.*
- *Make sure that the dyeing reagent is on ice and not exposed to light*
- *If negative control settings are required, set the sterilized distilled water to 36 μL, excluding the rTdT enzyme.*

8. Staining: Remove the remaining PBS from the cells, and add the staining reagent (50 μL per 5 cm^2)

Cover the coverslip, and place the slide in a humidified chamber at 37 ℃ for 1 h in a dark.

9. Quenching staining: 2x rinse buffer, prepared from 10-fold dilution of 20x rinse buffer, is added to a 50 mL conical tube. The staining slide is immersed into 2x rinse buffer tube for 15 min at 25 ℃.
10. Cleaning: Immerse the slides in a 50 mL conical tube containing PBS for 5 min. Remove unreacted dye-dUTP by repeating the procedure two more times.

• PI staining

① Fill a 50 mL conical tube with a PI solution diluted in 1 μg/mL in PBS, stand it in for 15 min at 25 ℃ in a dark.

② After dyeing, immerse the slide in distilled water for 5 min at 25 ℃. Replace the distilled water and repeat it three times.

③ Dry the slide.

④ Analyze with a microscope.

• Paraffin-embed tissue

This method is based on tissue sections after formalin fixation or paraffin treatment.

1. Cleaning: Immersing the slides in a 50 mL conical tube containing xylene for 5 min to remove the paraffin attached to the slide, replace xylene, and wash one more time.

   After that, the slide is immersed into a 50 mL conical tube containing 100% ethanol for 5 min at rt.
2. Rehydration: The slides are immersed into a conical tube containing in sequence of 100, 95, 85, 70, and 50% ethanol for 3 min at rt.
3. Primary fixation: Immerse the slides in a 50 mL conical tube containing PBS for 5 min and place slide in a 50 mL conical tube containing 4% paraformaldehyde in PBS (pH 7.4) for 15 min.
4. Cleaning: Immerse the slides in a 50 mL conical tube containing PBS for 5 min, replace with fresh PBS and repeat one more time.
5. Permeabilization: Remove the solution from the tissue sections and place them on a flat surface.

   Cover tissue sections with 100 μL of 20 μg/mL proteinase K per slide, and stand at rt for 8 to 10 min.

Note: Protease K treatment time should be optimized, if the tissue section thickness exceeds 4 to 6 μm, the treatment time should be increased, but excessive proteinase K treatment time may damage the tissue.

6. Secondary Fixation: Immerse the slides in a 50 mL conical tube containing PBS for 5 min at rt, and place the slides in a 50 mL conical tube containing 4% paraformaldehyde in PBS (pH 7.4) for 5 minutes.
7. Cleaning: The slides are immersed in a 50-mL conical tube containing PBS solution 5 min, and the PBS is replaced one more time.

If positive control is required, prepare at this stage

• Positive control

① Cover the fixed cells with 100 μL of DNase I buffer, and incubate at 25 ℃ for 5 minutes.

② Remove the DNase I buffer. Cover slide with 100 μL of DNase I buffer containing DNase I A (5.5 ~ 10 unit/mL) and incubate at 25 ℃ for 10 min.

③ Shake the slides gently to discard the solution, and place slides into a 50 mL conical tube containing distilled water and wash 3 or 4 times.

8. Buffer Equilibration: Remove the PBS on the slide as much as possible by tapping the slides, cover the cells with 100 μL of 1x reaction buffer, 5-fold diluted 5X buffer, and incubate at rt for 5-10 min.
9. Staining solution preparation: Prepare the staining reagent during the process.

*prepare a sufficient amount of dye reagent, including positive control, refer below table.

Note:

■ *1 assay is based on 5 cm^2 of sample area. If the area is larger than that, the amount of reagents should be increased in proportional to the area.*

■ *Make sure that the dyeing reagent is on ice and not exposed to light*

If negative control settings are required, set the sterilized distilled water to 36 μL, excluding the rTdT enzyme

10. Staining: Remove the remaining PBS from the cells, and add the staining reagent (50 μL per 5 cm^2)
Cover with coverslip and place the slide in a humidified chamber at 37 ℃ for 1 h in a dark.
11. Quenching staining: 2x rinse buffer, prepared from 10-fold dilution of 20x rinse buffer, is added to a 50 mL conical tube. The staining slide is immersed into 2x rinse buffer tube for 15 min at 25 ℃.
12. Cleaning: Immerse the slides in a 50 mL conical tube containing PBS for 5 min. Remove unreacted dye-dUTP by repeating the procedure two more times.

• PI staining

① Fill a 50 mL conical tube with a PI solution diluted in 1 μg/mL in PBS, stand it in for 15 min at 25℃ in a dark.

② After dyeing, immerse the slide in distilled water for 5 min at 25 ℃. Replace the distilled water and repeat it three times.

③ Dry the slide.

④ Analyze with a microscope.

Custom Labeling Service

Based on accumulated know-how and technologies, BioActs provide a wide range of custom services such as protein fluorescence labeling, organic synthesis, oligonucleotide synthesis upon customers' request. Our reliable technology has acknowledged by our clients from domestic and overseas universities, institutions, in vitro diagnostic and pharmaceutical companies and has enabled to steadily conduct their requirements. In addition, we can introduce fluorescent materials to many other compounds such as organic and inorganic compounds, drugs, hormones, polymer, peptides, proteins, antibodies, etc. We also can provide chemical and optical analytical data, along with cell and animal experiments.

Technical Support

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SDS (Safety Data Sheets) You can find SDS at www.bioacts.com, the official website of BioActs.
CoA (Certificate of Analysis) provides detailed quality information of each product. To see CoA, check the lot number written on each product's page at www.bioacts.com, when having trouble with check, contact to our technical support team

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The product cannot be resold to other regions except for South Korea.
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