Apoptosis DNA Ladder Extraction Kit

Introduction

Apoptosis refers to the process of programmed cell death that occurs in response to specific signals, involving a series of biochemical changes. When cells detect stress signals, cysteine proteases (caspases) are activated, which in turn activate Caspase-Activated DNase (CAD), leading to the degradation of DNA into fragments of 180-200 bp or their multiples. DNA fragmentation is one of the key markers of apoptosis.

Apoptosis DNA Ladder Extraction Kit is specifically designed for extracting fragmented DNA produced during apoptosis. It efficiently extracts DNA fragments as small as 180-200 bp, while also capable of isolating genomic DNA larger than 50 kb.

Components and Storage

Size / Components	K1629-50 tests	Storage
Lysis Buffer	30 mL	-20℃
Enzyme A Solution	1vial	-20℃
Enzyme B Solution	0.25 mL	-20℃
10mM Ammonium acetate	6 mL	-20℃
TE Buffer	6 mL	-20℃
Shipping: Blue Ice	**Shelf life:** 12 months	

Protocol

1. **Experimental Preparation:** Tris-saturated phenol pH 8.0; isopropanol; chloroform; anhydrous ethanol.
2. Add 275 μL of ddH_2O to the Enzyme A Solution, mix thoroughly, and aliquot for storage at -80°C.
3. **Induce apoptosis:** Use the desired method to induce apoptosis in the cells, while setting up control cultures to observe the uninduced condition.
4. **Sample Collection:**

   Suspension Cell Samples: Wash the cells with PBS (not provided), centrifuge at 1000-1500 g for 5 minutes, and discard the supernatant to collect the cell pellet.

   Adherent Cell Samples: After gentle digestion with trypsin, wash with PBS 1-2 times, centrifuge at 1000-1500 g for 5 minutes, and discard the supernatant to collect the cell pellet.

Tissue Samples: Cut the tissue into very small pieces, homogenize in PBS to generate a cell suspension (do not sonicate), and centrifuge at 1000-1500 g for 5 minutes to collect the cell pellet.

5. DNA Ladder Extraction

1) Take 5 mg of tissue or 10^6 cells, resuspend the sample in 500 µL of Lysis Buffer, and incubate on ice for 2-5 minutes. Then centrifuge at 12,000 g for 2 minutes at 4°C, and transfer the supernatant to a new EP tube.

***Note:** Gently pipette the sample.

2) Add 5 µL of Enzyme A Solution to each sample, gently vortex to mix, and incubate at 56°C for 15 minutes.

3) Add 5 µL of Enzyme B Solution to each sample, gently vortex to mix, and incubate at 37°C for 2 hours.

4) For every 500 µL of sample, add an equal volume of Tris-saturated phenol pH 8.0 and mix thoroughly. Then centrifuge at 12,000 g for 5 minutes at 4°C, carefully take out approximately 300 µL of upper phase liquid and transfer it to a new EP tube.

***Note:** a. To avoid protein contamination in the experiment, do not aspirate the interphase.

b. Phenol is toxic, please use it in a fume hood for safety.

5) If further improvement in separation efficiency is required, repeat the operation in **step 5.4** on the collected upper phase liquid 1-2 times.

6) Take approximately 300 µL of upper phase liquid, add 60 µL of 10M Ammonium acetate and 600 µL of ice-cold absolute ethanol, mix thoroughly by inversion, and incubate the sample at -20°C for 1 hour.

***Note:** a. Overnight storage at -70°C yields better results.

b. Using ice-cold absolute ethanol provides better precipitation.

7) Centrifuge at 12,000 g for 10 minutes at 4°C, remove the supernatant to obtain the desired DNA pellet.

8) Add 600 µL of freshly prepared ice-cold 70% ethanol, gently mix by inversion about 2 times. Centrifuge at 12,000 g for 10 minutes at 4°C, and carefully remove the supernatant.

***Note:** a. Using ice-cold ethanol provides better precipitation.

b. Care must be taken during the 70% ethanol washing step to avoid losing small DNA pellets, as these pellets contain most of the required DNA ladder.

9) Open the lid and air dry for about 1-2 minutes to remove any residual ethanol as much as possible, in order to avoid any impact on subsequent experimental operations.

10) Add 50 µL-100 µL of TE Buffer to dissolve the DNA pellet, mix thoroughly, and store at -20°C.

11) Take a portion of the DNA, mix with DNA Loading Buffer (**Cat. No.: K1161, K1162**), and take 15-30 µL of the sample for 1% agarose gel electrophoresis analysis. In the case of apoptosis, a typical DNA ladder will be visible.

***Note:** a. It is essential to use freshly prepared electrophoresis buffer during electrophoresis, and ensure that the DNA gel is also prepared with fresh solution, preferably used within 2 hours;

b. Recommended electrophoresis conditions are 20 V/cm for 3 hours; alternatively, one can start with an electrophoresis at 200 V/cm for 5 minutes, then adjust to 60 V/cm for 1 hour.

Note

1. Experimental procedures must be performed in a DNase-free environment to prevent DNA degradation.
2. If the DNA pellet is difficult to dissolve, it can be placed at 4°C and gently shaken overnight to aid in dissolution.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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