CYDN17-CHANGYU Selective Apoptotic DNA Ladder Extraction Kit

Catalog number	Package
CYDN1701	25 preps
CYDN1702	50 preps

Storage and shipping: Extraction buffer and Precipitant at 4 °C; 10% SDS at 15-25 °C; Enzyme A and Enzyme B at -20 °C. Enzyme B is supplied as a lyophilized powder for stability during transport; dissolve it in 500 µl (25 preps) or 1 ml (50 preps) of sterile water and store at -20 °C. Avoid repeated freeze-thaw cycles of Enzyme A and Enzyme B.

Applications

For rapid extraction of apoptotic DNA ladder.

Kit components

Component	Storage	25 preps (CYDN1701)	50 preps (CYDN1702)
Extraction buffer	4 °C	5 ml	10 ml
10% SDS	15-25 °C	500 µl	1 ml
Enzyme A	-20 °C	500 µl	1 ml
Enzyme B	-20 °C	500 µl	1 ml
Precipitant	4 °C	3.5 ml	7 ml

Description

A striking morphological feature of apoptotic or programmed cell death is the regular cleavage of chromosomal DNA at nucleosome intervals (185 bp) into fragments of about n × 185 bp (n = 1, 2, 3, 4...). On agarose gel electrophoresis these fragments appear as a ladder-like apoptotic DNA ladder, the most direct hallmark of apoptotic cells. This kit selectively isolates apoptotic DNA ladder from tissues and cells. By selectively separating genomic DNA from the apoptotic DNA ladder, interference of genomic DNA with observation of the ladder is minimized and detection sensitivity is markedly improved. The reaction is carried out in microcentrifuge tubes and is completed in 2.5 hours; it is fast and convenient, requires no organic extraction and is highly sensitive, detecting a DNA ladder from about 2,000 apoptotic cells. A starting amount of 5-10 × 10^5 cells is recommended, but 1 × 10^5 to 5 × 10^6 cells may be used. As a rule, the total cell population should contain at least about 1-2 × 10^4 apoptotic cells; more than 2 × 10^4 apoptotic cells usually gives a very clear apoptotic DNA ladder. The kit can also be used to extract apoptotic DNA ladder from tissues. However, compared with cultured cells, apoptosis in whole-animal tissues is less regular in timing, location and extent, which often makes accurate sampling difficult and may significantly affect the results; an experienced user can still extract apoptotic DNA ladder from tissue provided that apoptosis has indeed occurred (see Product note 4).

Notes

Note: Enzyme B is shipped as a lyophilized powder. After receipt, add 500 µl of sterile water (25 preps) or 1 ml of sterile water (50 preps) to dissolve it, then store at -20 °C.

Note: Enzyme A and Enzyme B are enzyme solutions; avoid repeated freeze-thaw cycles, which reduce activity. If the kit is to be used several times, aliquot each enzyme according to the amount needed per use and store the aliquots at -20 °C.

Product notes

1. Over-staining with ethidium bromide reduces the sensitivity of DNA band detection; the gel can be rinsed with water for 10-30 minutes. If it has been rinsed too long, the gel can be restained with ethidium bromide. A more sensitive DNA stain such as SYBR Green may also be used. Polyacrylamide DNA gel electrophoresis and DNA silver staining are also possible.

2. After treatment of cells, apoptosis may be most obvious only at a certain time point or a certain treatment intensity; a preliminary experiment is needed to determine the optimal induction time or intensity. The Apoptotic Body / Hoechst Staining Kit (CYDN1801) can also be used for rapid staining of apoptotic bodies.

3. A starting amount of 5-10 × 10^5 cells is recommended, but 1 × 10^5 to 5 × 10^6 cells may be used. As a rule, the total cell population should contain at least about 1-2 × 10^4 apoptotic cells; more than 2 × 10^4 apoptotic cells usually gives a very clear apoptotic DNA ladder. One well of a six-well plate corresponds to one 35 mm culture dish, and a confluent culture yields 1-10 × 10^5 cells; if the apoptosis rate is 10%, treatment yields about 1-10 × 10^4 apoptotic cells, which should be sufficient for a clear apoptotic DNA ladder. Conversely, if no clear apoptotic DNA ladder can be obtained from more than 3 × 10^6 cells, fewer than 1% of the cells are apoptotic, and increasing the cell amount is unlikely to help.

4. For extraction of apoptotic DNA ladder from tissue blocks: place 10-20 mg of tissue in a small glass homogenizer, add 100-200 µl of Extraction buffer and homogenize manually up and down 15-20 times. Remove the homogenate and keep on ice for 5-10 minutes. Vortex for 10 seconds. Centrifuge at 4,500 rpm for 10 minutes, collect the supernatant into a new 1.5 ml centrifuge tube and continue with extraction step 3. Alternatively, mince 30-50 mg of tissue and homogenize in PBS to prepare a cell suspension; collect the cells by centrifugation and continue with the procedure from step 2.

5. Use high-quality agarose, a narrow and thin sample comb and a relatively thin agarose gel (about 2-4 mm thick); run slow electrophoresis at a low voltage. This markedly increases the detection sensitivity of apoptotic DNA bands. Do not run the electrophoresis distance too long, otherwise the small apoptotic DNA bands diffuse and resolution decreases.

❖ Procedure

1. Wash the cells twice with PBS, collect 5-10 × 10^5 cells by centrifugation at 500 ×g at 4 °C for 5 minutes (preferably include a non-apoptotic cell control). Carefully aspirate the supernatant with a pipette and remove any liquid adhering to the tube wall.

2. Gently loosen the cell pellet at the bottom of the tube by flicking it with a finger, add 100 µl of Extraction buffer, mix vigorously on a vortexer for 10 seconds and centrifuge at 1,100-1,600 ×g (about 3,500-4,500 rpm) for 5 minutes.

3. Without disturbing the pellet, transfer the supernatant to a new 1.5 ml centrifuge tube.

4. Treat the pellet again as in step 2.

5. Combine the supernatant with that from step 3 (about 200 µl in total) as the crude extract, which contains apoptotic DNA fragments while non-apoptotic chromosomal DNA has been removed by precipitation.

6. Add 20 µl of 10% SDS solution and then 20 µl of Enzyme A to the crude extract, mix and incubate at 56 °C for 1 hour.

7. Add 20 µl of Enzyme B, mix and incubate at 37 °C for 1 hour, or until the solution becomes clear (may be left overnight).

8. Add 130 μl of Precipitant, mix by inversion, then add 1 ml of ethanol, mix and keep at -20 °C for at least 1 hour to precipitate apoptotic DNA fragments.

9. Centrifuge at at least 13,000 rpm at 4 °C for 15 minutes, discard the supernatant, wash once with 1 ml of 70% ethanol, centrifuge, pour off the ethanol and remove as much liquid adhering to the tube wall as possible. Leave the tube open and air-dry the pellet at room temperature.

10. Dissolve the pellet thoroughly in 17 μl of double-distilled water or TE buffer, add 3 μl of 6× DNA gel loading buffer and vortex to mix. Load all 20 μl or an appropriate amount on a 1% agarose gel, stain with ethidium bromide and observe and photograph under UV. When the apoptosis rate is low, dissolving the pellet in an excess of TE buffer may dilute the sample too much to detect the DNA ladder, so a smaller volume may be used; conversely, a larger volume may be used when apoptosis is extensive.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.