Apoptosis DNA fragmentation analysis protocol

A distinctive biochemical feature of apoptosis is the fragmentation of DNA by a specific nuclease called caspase-activated DNase (CAD). Activation of CAD by the caspase cascade leads to specific cleavage of the DNA at the internucleosomal linker sites, generating fragments of ~200 base pairs known as DNA ladders.

The classical method to detect DNA ladders is to examine fragmented genomic DNA on an agarose gel. This semi-quantitative method is a simple technique that provides a robust answer.

Harvest cells

1. Pellet cells
2. Lyse cells in 0.5 mL detergent buffer: 10 mM Tris (pH 7.4), 5 mM EDTA, 0.2% Triton
3. Vortex
4. Incubate on ice for 30 min
5. Centrifuge at 27,000 x *g* for 30 min
6. Divide supernatants into two 250 µL aliquots
7. Add 50 µL ice-cold 5 M NaCl to each aliquot and vortex

Precipitate DNA

1. Add 600 µL ethanol and 150 µL 3 M sodium acetate, pH 5.2 and mix by pipetting up and down.
2. Incubate tubes at -80°C for 1 h.
3. Centrifuge 20,000 x *g* for 20 min; discard supernatants carefully.
4. Pool DNA extracts together by re-dissolving the pellets in a total of 400 µL extraction buffer (10 mM Tris and 5 mM EDTA).
5. Add 2 µL of 10 mg/mL DNase-free RNase and incubate for 5 h at 37°C.
6. Add 25 µL proteinase K at 20 mg/mL and 40 µL of buffer (100 mM Tris pH 8.0, 100 mM EDTA, 250 mM NaCl
7. Incubate overnight at 65°C.
8. Extract DNA with phenol/chloroform/isoamyl alcohol (25:24:1) and precipitate with ethanol.
9. Carefully discard supernatant trying not to disturb the pellet as it is quite loose.

Load DNA in agarose gel

1. Air-dry pellet and resuspend in 20 µL Tris-acetate EDTA buffer supplemented with 2 µL of sample buffer (0.25% bromophenol blue, 30% glycerol)
2. Separate DNA electrophoretically on a 2% agarose gel containing 1 µg/mL ethidium bromide and visualize by ultraviolet transillumination

Protocol tips

- The DNA will make the sample very viscous and sticky. Use the DNA sample loading buffer at a higher concentration than you normally would to ensure the sample does not float away from the well.
- Prepare an agarose gel with 1.8–2 % agarose content. The high agarose concentration provides the necessary resolution to see the steps in the ladder.
- Run the gel at a lower voltage for a longer time than you normally would to avoid overheating and subsequent deformation of the DNA bands.

Protocol edited from procedure kindly provided by: Dr Richard Pattern, Tufts-New England Medical Center, US.