Apoptotic DNA Ladder Extraction Kit

(Catalog #K170-50; 50 assays; Store at –20°C)

I. Introduction:

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. BioVision's **Apoptotic DNA Ladder Extraction Kit** provides an easy and sensitive means for detecting DNA fragmentation in apoptotic cells. The new procedure selectively extract DNA ladders without interference of intact genomic DNA, which significantly increase the cell numbers that can be can be extracted and load on one agarose gel well, therefore increase the detection sensitivity. The kit can extract apoptotic DNA ladder from both tissues and cells with as little as 5% or less apoptotic cell population. The extracted apoptotic DNA ladder fragments can be easily visualized by agarose gel electrophoreses.

II. Kit Contents:

Component	K170-50 50 Assays	Color Code Cap Color	Part Number
DNA Ladder Extraction Buffer	12.5 ml	WM	K170-50-1
Enzyme A Solution	0.25 ml	Blue	K170-50-2
Enzyme B (Lyophilized)	1 vial	Red	K170-50-3
Ammonium Acetate Solution	0.25 ml	Yellow	K170-50-4
DNA Suspension Buffer	2 ml	Green	K170-50-5

III. Reagent Preparation:

- Dissolve Enzyme B with 275 µl ddH_2O and mix well before use. The Enzyme B solution should be aliquoted and freeze at –70°C immediately.

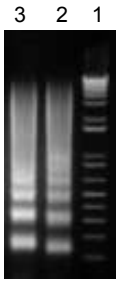
II. DNA Ladder Detection Protocol:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Wash cells with PBS (not provided) and pellet 2 x 10^6 cells by centrifugation for 5 min at 500 x g. Carefully remove supernatant using pipette.

   For adherent cells, gently trypsinize cells and then pellet cells.

   For tissue samples, cut 50 mg tissues into very fine pieces or homogenize tissues in PBS to generate cell suspension (Note: do not sonicate). Centrifuge to collect cell pellet.

   Note: The kit can detect DNA ladder from 10^5 apoptotic cells (100% apoptosis). However, if the level of apoptosis in your sample is low, you can increase the cell number up to 10^7. If using more than 2 x 10^6 cells per assay, you should proportionally increase the volume of all reagents.
3. Extract the cell pellet with 50 µl DNA Ladder Extraction Buffer for 10 seconds at room temperature with gentle pipetting. Centrifuge for 5 min at 1600 x g (~4500 rpm). Transfer the supernatant to a fresh tube.
4. Extract the pellet again by repeating step 3. Combine the supernatant.
5. Add 5 µl Enzyme A Solution into the supernatant, mix by gentle vortex and incubate at 37°C for 10 min. (Note: If cells contain high level of DNase, then the incubation step should be skipped, as high level DNase can digest DNA ladder generating smear pattern.)
6. Add 5 µl Enzyme B Solution into each sample and incubate at 50 °C for 30 min or longer (overnight is ok).
7. Add 5 µl Ammonium Acetate Solution to each sample and mix well. Add 100 µl isopropanol (not provided), mix well, and keep at –20°C for 10 minutes.
8. Centrifuge the sample at maximum speed (~16K x g) for 10 minutes to precipitate DNA. (**Note: Microcentrifuges typically generate ~ 16K x g at 13K x rpm)**
9. Remove supernatant, wash the DNA pellet with 0.5 ml 70% ethanol, centrifuge again at maximum speed (~16K x g) to remove trace ethanol, and air dry for 10 minutes at room temperature.
10. Dissolve the DNA pellet in 30 µl DNA Suspension Buffer (Note: No other loading buffer needed for loading to the gel).
11. Load 15-30 µl of the sample onto a 1.2% agarose gel containing 0.5 µg/ml ethidium bromide in both gel and running buffer.
12. Run the gel at 5 V/cm for 1-2 hours or until the yellow dye (included in the suspension buffer) run to the edge of the gel.
13. Ethidium bromide-stained DNA can be visualized by trans-illumination with uv light and photographed.



DNA ladders were extracted from 2x10^6 Jurkat cells treated with 2 µM Camptothes, according to kit instructions. The kit extracts laddered DNA only, not the intact genomics, therefore more cells can be extracted and load on the agarose gel.

Lane 1: 1 kb DNA ladder;
Lane 2: 6 hour induction;
Lane 3: 12 hour induction.

V. Related Products

Apoptosis Detection Kits & Reagents
- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- siRNA Apoptosis Vectors

Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence
- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit