

Enhanced Apoptotic DNA Ladder Detection Kit

(Catalog #K130-50; 50 assays; Store at -20°C)

I. Introduction:

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. BioVision's **Enhanced Apoptotic DNA Ladder Detection Kit** provides an easy and sensitive means for detecting DNA fragmentation in apoptotic cells. Unlike other commercially available kits that require 1-2 days to perform the procedure, the new detection method requires less than 90 minutes to prepare DNA, with neither extraction nor using columns. DNA fragmentation can be easily visualized by agarose gel electrophoreses stained with a highly sensitive dye. The new procedure provides higher sensitivity in comparison to other similar kits in the market. The assay can be used to detect apoptotic DNA ladder in both tissues and cells.

II. Kit Contents:

Component	K130-50	Color Code	Part Number
	50 Assays	Cap Color	
TE Lysis Buffer	1.8 ml	Purple	K130-50-1
Enzyme B (Lyophilized)	1 vial	Red	K130-50-2
Ammonium Acetate Solution	0.25 ml	Yellow	K130-50-3
DNA Suspension Buffer	0.25 ml	Green	K130-50-4
Staining Dye (10000X)	50 µl	Blue	K130-50-5

III. Reagent Preparation:

- Dissolve Enzyme B with 275 µl ddH₂O and mix well before use. The Enzyme B solution should refreeze at -70°C immediately after each use, or aliquot and then stored at -70°C for future use.
- Staining Dye (10000X) should be diluted to 1X with TAE or TE buffer (not provided) just before use in Step 12 (For each staining, dilute 5 µl to 50 ml).

IV. DNA Ladder Detection Protocol:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Pellet 5-10 x 10⁵ cells in a 1.5 ml microcentrifuge tube.
3. Wash cells with PBS (not provided) and pellet cells by centrifugation for 5 min at 500 x g. Carefully remove supernatant using pipette.
4. Lyse cells with 35 µl TE Lysis Buffer, gentle pipetting, incubate at 37°C for 10 min. Note: If cells contain high level of DNase, the incubation step should be skipped, as high level DNase can digest DNA ladder generating smear pattern.
5. Add 5 µl Enzyme B Solution into each sample and incubate at 50°C for 30 min or longer (overnight is ok).
6. Add 5 µl Ammonium Acetate Solution to each sample and mix well. Add 50 µl isopropanol (not provided), mix well, and keep at -20°C for 10 minutes.
7. Centrifuge the sample for 10 minutes to precipitate DNA.
8. Remove supernatant, wash the DNA pellet with 0.5 ml 70% ethanol, remove trace ethanol, and air dry for 10 minutes at room temperature.
9. Dissolve the DNA pellet in 20 µl DNA Suspension Buffer.
10. Load the sample onto a 1.8% agarose gel.

11. 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.
12. Stain the gel with 50 ml 1X Staining Buffer (1:10000 dilution of the stock Staining Dye in 1X TAE or TE buffer) for at least 30 minutes with gentle shaking of the gel.
13. DNA ladder can be visualized by illumination of short UV wavelength (254 nm) and photographed with camera equipped with 520 nm filter.

Note: The illumination time should be no more than 1 minute. Longer illumination may significantly decrease the signal.

V. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents

- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA 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
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

Cell Damage & Repair

- HDAC & HAT Fluorometric & Colorimetric Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

Signal Transduction

- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP & PLTP Activity Assay & Drug Discovery Kits
- Cholesterol Quantification Kit

Molecular Biology & Reporter Assays

- siRNA Expression Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits

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