Quick Apoptotic DNA Ladder Detection Kit

(Catalog #BN00064; 50 assays; Store at –20°C)

I. Introduction:

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. Assay Genie's **Quick 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. The new procedure increases recovery of small fragmented DNA, and therefore improves the sensitivity of the assay.

II. Kit Contents:

Component	BN00064 50 Assays	Color Code Cap Color
TE Lysis Buffer	1.8 ml	Purple
Enzyme A Solution	0.25 ml	Blue
Enzyme B (Lyophilized)	1 vial	Red
Ammonium Acetate Solution	0.25 ml	Yellow
DNA Suspension Buffer	1.5 ml	Green

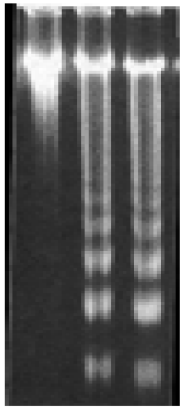
III. Reagent Preparation:

- Dissolve Enzyme B with 275 µl ddH_2O 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.

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 10^5 cells in a 1.5 ml microcentrifuge tube.

   Note: For adherent cells, gently trypsinize cells and then pellet cells.
3. Wash cells with PBS (not provided) and pellet cells by centrifugation for 5 min at 500 xg. Carefully remove supernatant using pipette.
4. Lyse cells with 35 µl TE Lysis Buffer, gentle pepitting.
5. Add 5 µl Enzyme A Solution, 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 50 µl isopropanol (not provided), mix well, and keep at –20°C for 10 minutes.
8. Centrifuge the sample for 10 minutes to precipitate DNA.
9. Remove supernatant, wash the DNA pellet with 0.5 ml 70% ethanol, remove trace ethanol, and air dry for 10 minutes at room temperature.
10. Dissolve the DNA pellet in 30 µl DNA Suspension Buffer.
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.



Quick Detection of Apoptotic DNA Ladder in Jurkat Cells. Apoptosis was induced in Jurkat cells with camptothecin (2 µM) for 0 hr (Lane 1), 6 hrs (Lane 2) and 12 hrs (Lane 3). Chromosomal DNA was prepared using the Quick Apoptotic DNA Ladder Detection Kit according to the kit instructions. 20 µl of each sample was electrophoresed on a 1.2% agarose/EtBr gel.

FOR RESEARCH USE ONLY! Not to be used on humans.