



## Quick Apoptotic DNA Ladder Detection Kit

*Manufactured by BioVision.*

**ALX-850-242-KI01**

~50 tests

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**For laboratory use only. Not for human or diagnostic use.**

## I. Introduction:

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

## II. Kit Contents:

Components	850-242-KI01	Color code
	50 tests	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	2 ml	Green

## III. Reagent Preparation:

- Dissolve Enzyme B with 275  $\mu$ l distilled water and mix well before use. The Enzyme B solution should be refrozen at  $-80^{\circ}\text{C}$  immediately after each use, or aliquoted and stored at  $-80^{\circ}\text{C}$ .

## 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<sup>5</sup> cells in a 1.5 ml microcentrifuge tube.  
**Note:** For adherent cells, gently trypsinize cells and then pellet.
3. Wash cells with PBS (not provided) and pellet cells by centrifugation for 5 minutes at 500 x g. Carefully remove supernatant using a pipette.
4. Lyse cells with 35  $\mu$ l TE Lysis Buffer, gently pipetting.
5. Add 5  $\mu$ l Enzyme A Solution, mix by gently vortexing and incubate at 37 $^{\circ}\text{C}$  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 and generate a smear pattern.
6. Add 5  $\mu$ l Enzyme B Solution into each sample and incubate at 50 $^{\circ}\text{C}$  for 30 min. or longer (overnight is ok).
7. Add 5  $\mu$ l Ammonium Acetate Solution to each sample and mix well. Add 50  $\mu$ l isopropanol (not provided), mix well and keep at  $-20^{\circ}\text{C}$  for 10 min.
8. Centrifuge the sample for 10 min. to precipitate DNA.

9. Remove supernatant, wash the DNA pellet with 0.5 ml of 70% ethanol; remove trace ethanol and air dry for 10 min. at room temperature.
10. Dissolve the DNA pellet in 30  $\mu$ l DNA Suspension Buffer.
11. Load 15-30  $\mu$ l of the sample onto a 1.2% agarose gel containing 0.5  $\mu$ g/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) runs to the edge of the gel.
13. Ethidium bromide-stained DNA can be visualized by trans-illumination with UV light and photographed.

**V. Storage and Stability:**

- Store kit at  $-20^{\circ}\text{C}$ . Avoid freeze/thaw cycles.
- Store Enzyme B solution at  $-80^{\circ}\text{C}$ .