

ab66090

Apoptosis DNA Ladder Assay Kit

Instructions for Use

For the rapid, sensitive and accurate detection of DNA fragmentation in apoptotic cells.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

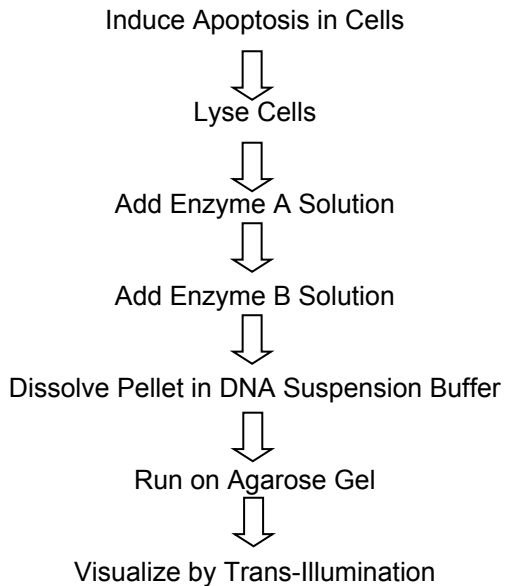
1. Overview	3
2. Protocol Summary	4
3. Components and Storage	5
4. Assay Protocol	7
5. Data Analysis	9

1. Overview

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. Abcam's Apoptosis DNA Ladder Assay 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
TE Lysis Buffer	1.8 mL
Enzyme A Solution	0.25 mL
Enzyme B (Lyophilized)	1 vial
Ammonium Acetate Solution	0.25 mL
DNA Suspension Buffer	1.5 mL

* Store kit at -20°C.

ENZYME B SOLUTION: Dissolve Enzyme B with 275 μ l ddH₂O and mix well before use. The Enzyme B solution should be frozen at -80°C immediately after each use, or aliquot and then stored at -80°C for future use.

B. Additional Materials Required

- Microcentrifuge
- Isopropanol
- Pipettes and pipette tips
- Orbital shaker
- 70% ethanol
- 1.2% agarose gel containing 0.5 μ g/ml ethidium bromide

4. Assay 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.

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 x *g*. Carefully remove supernatant using pipette.
4. Lyse cells with 35 μ l TE Lysis Buffer, gentle pipetting.
5. Add 5 μ l Enzyme A Solution, mix by gentle vortex and incubate at 37°C for 10 min.

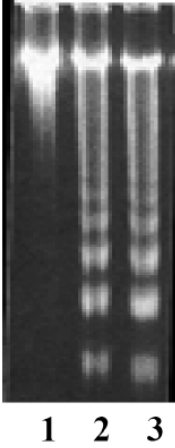
Note:

If cells contain high levels of DNase, then the incubation step should be skipped, as high level DNase can digest DNA ladder generating smear pattern.

6. Add 5 μ l Enzyme B Solution into each sample and incubate at 50°C for 30 min or longer (overnight is OK).
7. Add 5 μ l Ammonium Acetate Solution to each sample and mix well. Add 50 μ l 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 μ l DNA Suspension Buffer.
- 11.** Load 15-30 μ l of the sample onto a 1.2% agarose gel containing 0.5 μ 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) run to the edge of the gel.
- 13.** Ethidium bromide-stained DNA can be visualized by trans-illumination with UV light and photographed.

5. Data Analysis



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 Apoptosis DNA Ladder Assay Kit according to the kit instructions. 20 μl of each sample was electrophoresed on a 1.2% agarose/EtBr gel.

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