ab65627

Apoptotic DNA Ladder Isolation 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.

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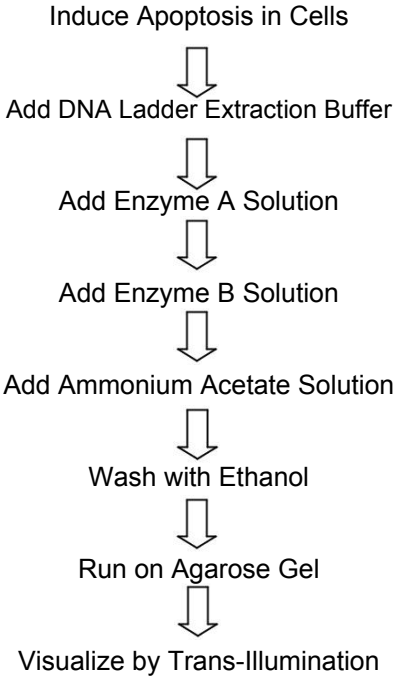
1. Overview

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. Abcam's Apoptotic DNA Ladder Isolation Kit provides an easy and sensitive means for detecting DNA fragmentation in apoptotic cells.

The new procedure selectively extracts DNA ladders without interference of intact genomic DNA, which significantly increases the cell numbers that can be can be extracted and loaded on one agarose gel well, therefore increasing the detection sensitivity.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
DNA Ladder Extraction Buffer	12.5 mL
Enzyme A Solution	0.25 mL
Enzyme B (Lyophilized)	1 vial
Ammonium Acetate Solution	0.25 mL
DNA Suspension Buffer	2 mL

* Store kit at -20°C.

Dissolve Enzyme B with 275 µl ddH_2O and mix well before use. The Enzyme B solution should be aliquoted and frozen at -80°C immediately.

B. Additional Materials Required

- Microcentrifuge
- PBS
- Isopropanol
- 70% Ethanol
- Pipettes and pipette tips
- Orbital shaker
- 1.2% agarose gel containing 0.5 µg/ml ethidium bromide

4. Assay 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.
   a. **For adherent cells:** Gently trypsinize cells and then pellet cells.
   b. **For tissue samples:** Cut 50 mg tissues into very fine pieces or homogenize tissues in PBS to generate cell suspension **(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 a smear pattern.

6. Add 5 µl Enzyme B Solution into each sample and incubate at 50°C for 30 min or longer (overnight is acceptable).

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 (~16,000 x g) for 10 minutes to precipitate DNA.

Note:

Microcentrifuges typically generate ~16,000 x g at 13,000 x rpm

9. Remove supernatant, wash the DNA pellet with 0.5 ml 70% ethanol, centrifuge again at maximum speed (~16,000 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:

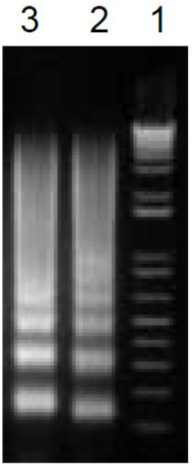
No other loading buffer is needed for loading the gel.

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.

5. Data Analysis



Isolation of Apoptotic DNA Ladder in Jurkat

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Cells: DNA ladders were extracted from 2x10 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 2: 6 hour induction

Lane 3: 12 hour induction

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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