Enhanced Apoptotic DNA Ladder Detection Kit

Instructions for Use

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

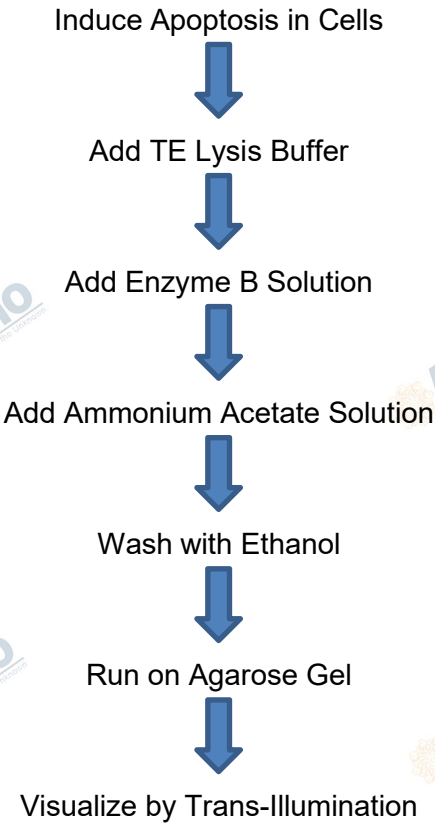
This product is for research use only and is not intended for diagnostic use.

1. Overview

Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. APExBIO'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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
TE Lysis Buffer	1.8 mL
Enzyme B (Lyophilized)	1 vial
Ammonium Acetate Solution	0.25 mL
DNA Suspension Buffer	0.25 mL
Staining Dye (10000X)	50 µL

* Store kit at -20°C.

ENYZME B SOLUTION: 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 after each use, or aliquoted and then stored at -80°C for future use.

STAINING DYE SOLUTION: 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).

B. Additional Materials Required

- Microcentrifuge
- Isopropanol
- 70% Ethanol
- Pipettes and pipette tips
- Orbital shaker
- 1.8% agarose gel

4. Assay 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.
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 pepitting, 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) runs 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.

For research use only! Not to be used in humans.

For more details, please visit http://www.apexbt.com/or contact our technical team.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com