Quick Apoptotic
DNA Ladder Detection Kit
Catalog# KHO1021 (50 Tests)

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INTENDED USE

The Apo*Target*™ Quick Apoptotic DNA Ladder Detection Kit is designed for preparation of nucleic acids from mammalian cells to determine the level of DNA fragmentation of apoptotic cells.

For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.

PRINCIPLE OF THE METHOD

Internucleosomal DNA fragmentation is considered as a hallmark of apoptosis. During apoptosis, activated nucleases degrade the higher order chromatin structure of DNA into fragments of 50 to 300 kilobases and subsequently into small DNA pieces of about 200 base pairs in length. These DNA fragments can be extracted from cells and visualized by horizontal gel electrophoresis followed by ethidium bromide staining. The detection of DNA fragments by gel electrophoresis is one method to identify cells undergoing apoptosis.

The ApoTarget™ Quick Apoptotic DNA Ladder Detection Kit provides a simple and rapid procedure for extraction of chromosomal DNA. The procedure prepares DNA for use in the methods that detect DNA fragmentation in apoptotic cells. Unlike other kits which require 1 to 2 days to finish, this detection method only requires less than 90 minutes to prepare DNA in a single tube, without the need for extractions or column steps. DNA fragmentation can be easily visualized by agarose gel electrophoresis. This procedure increases recovery of small fragmented DNA and, therefore, improves the sensitivity of the assay.

REAGENTS PROVIDED

Note: Store kit at -20°C. All reagents are stable for 1 year under proper storage conditions.

1. TE Lysis Buffer (1.8 mL): Buffered solution containing Tris, EDTA and detergent.
2. Enzyme A Solution (0.25 mL): Enzyme in buffered solution containing Tris and NaCl.
3. Enzyme B (1 vial): Dissolve Enzyme B with 275 µL TE Lysis Buffer and mix well before use. Store at -20°C after reconstitution and keep thaw and freeze cycles to a minimum.
4. Ammonium Acetate Solution (0.25 mL).
5. DNA Suspension Buffer (2 mL): Contains Tris, glycerol and orange G.

REAGENTS NOT PROVIDED

1. Agarose and TBE buffer (1 L TBE buffer contains 5.4 g Tris, 2.8 g Boric acid, 2 mL of 0.5 M EDTA solution, pH 8.0).
2. PBS.
3. Ethidium Bromide.
4. Ethanol.
5. DNA ladder marker.
6. Microcentrifuge.
7. DNA electrophoresis equipment.
8. UV light source and camera.

PROCEDURAL NOTES/ LAB QUALITY CONTROL

1. When not in use, kit components should be stored at -20°C as indicated on vial or bottle labels.
2. Samples should be stored frozen.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange different reagent lots from various kits.
5. Do not use reagents beyond the expiration date of the kit.

WARNINGS AND PRECAUTIONS

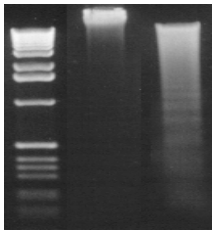
1. This kit is intended for research use only. It is not to be used for diagnostic procedures.
2. Never pipette by mouth.
3. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

ASSAY PROCEDURE

1. Induce apoptosis in cells by desired method. Concurrently incubate a control without induction. Pellet 5-10 x 10^5 cells in a 1.5 mL microcentrifuge tube.
2. Wash cells with PBS and pellet cells by centrifugation for 5 minutes at 500 x g.
3. Carefully discard the supernatant.
4. Lyse the cells with 35 µL TE Lysis Buffer by carefully pipetting up and down several times.
5. Add 5 µL Enzyme A Solution to the crude lysate. Mix by gentle vortexing and incubate at 37°C in a waterbath for 10 minutes.
6. Add 5 µL Enzyme B Solution to each sample and incubate at 50°C for 30 minutes in waterbath or until the lysate becomes clear.
7. Add 5 µL Ammonium Acetate Solution and 100 µL of absolute Ethanol (kept at -20°C) to each sample. Vortex and allow the DNA to precipitate at -20°C for 10 to 15 minutes.
8. Centrifuge the sample for 10 minutes at 12,000 to 14,000 rpm to collect the precipitated DNA.
9. Carefully discard the supernatant.
10. Add 0.5 mL of 70% cold ethanol to wash the DNA pellet and re-centrifuge the sample 10 minutes at 12,000 to 14,000 rpm.
11. Discard the supernatant and air-dry the DNA pellet for 10 minutes at room temperature.
12. Add 30 µL of DNA Suspension Buffer, and resuspend the DNA by carefully pipetting up and down several times.
13. Load 15 to 30 µL of each sample onto a 1.2% agarose gel containing 0.5 µg/mL ethidium bromide in both gel and running buffer (1x TBE).
14. Run the gel at 5 V/cm for 1 to 2 hours.
15. Ethidium bromide-stained DNA can be visualized by transillumination with UV light and photographed.

TYPICAL DATA

Apoptosis was induced in Jurkat cells by incubating cells with 2 µM Camptothecin for 5 hours at 37°C (Lane 3). Jurkat cells without Camptothecin were used as control (Lane 2). Lane 1 shows the DNA ladder marker. Detection of DNA fragmentation was done as described above.



1 2 3

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1. Wyllie, A.H. (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284(5756):555-6.
2. Park, D.J., *et al.* (1998) Detergent and enzyme treatment of apoptotic cells for the observation of DNA fragmentation. Biotechniques 24(4):558-60.