ApoTarget™ Quick Apoptotic DNA Ladder Detection Kit

Catalog Numbers KHO1021

Pub. No. MAN0004155 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Internucleosomal DNA fragmentation is a strong indicator of apoptosis. During apoptosis, activated nucleases degrade the higher order chromatin structure of DNA into fragments of 50 to 300 kilobases and then 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 that require 1 to 2 days to complete, 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 the recovery of small fragmented DNA, which improves the assay sensitivity.

Contents and storage

Item	Amount	Storage
TE Lysis Buffer [1]	1.8 mL	Store kit at –20°C.
Enzyme A Solution [2]	0.25 mL	
Enzyme B	1 vial	
DNA Suspension Buffer [3]	1.5 mL	
Ammonium Acetate Solution	0.25 mL	

[1] Buffered solution containing Tris, EDTA and detergent.
[2] Enzyme in buffered solution containing Tris and NaCl.
[3] Contains Tris, glycerol, and Orange G.

Required materials not supplied

- Agarose and TBE buffer (1 L of TBE buffer contains 5.4 g Tris, 2.8 g boric acid, and 2 mL of 0.5 M EDTA solution, pH 8.0)
- PBS
- Ethidium bromide
- Ethanol
- DNA ladder marker
- Microcentrifuge
- DNA electrophoresis equipment
- Ultraviolet (UV) light source and camera

Important product information

- Store samples frozen.
- Cover or cap all reagents when not in use.
- Do not mix or interchange different reagent lots from various kits.
- Do not use reagents after the expiration dates.

Before you begin

- Dissolve Enzyme B with 275 µL of TE Lysis Buffer and mix well before use. Store at –20°C after reconstitution. Do not perform multiple freeze/thaw cycles.

Assay procedure

1. Induce apoptosis in cells by the desired method. Concurrently incubate a control without induction. Centrifuge 5–10 x 10^5 cells in a 1.5 mL microcentrifuge tube.
2. Wash cells with PBS, then centrifuge at 500 x *g* for 5 minutes to form a pellet.
3. Carefully discard the supernatant.
4. Lyse the cells with 35 µL of TE Lysis Buffer by carefully pipetting up and down several times.
5. Add 5 µL of Enzyme A Solution to the crude lysate. Gently vortex to mix, then incubate in a water bath set at 37°C for 10 minutes.
6. Add 5 µL of Enzyme B Solution to each sample, then incubate in a water bath set at 50°C for 30 minutes or until the lysate becomes clear.

7. Add 5 µL of Ammonium Acetate Solution and 100 µL of absolute ethanol (at –20°C) to each sample. Vortex, then incubate at –20°C for 10–15 minutes to allow the DNA to precipitate.
8. Centrifuge the sample at 12,000–14,000 rpm for 10 minutes 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 centrifuge the sample at 12,000–14,000 rpm for 10 minutes.
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 15–30 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 1–2 hours.
15. View ethidium bromide-stained DNA using UV transillumination.

Example data

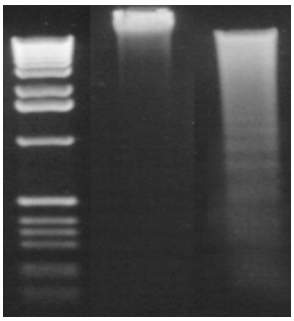

Fig. 1 Detection of DNA fragmentation using the ApoTarget™ Quick Apoptotic DNA Ladder Detection Kit.
The experiment was done as described in the assay procedure. (Lane 1) The DNA marker ladder is shown. (Lane 2) Jurkat cells without Camptothecin were used as a control. (Lane 3) Apoptosis was induced in Jurkat cells by incubating cells with 2 µM Camptothecin for 5 hours at 37°C. Jurkat cells without Camptothecin were used as a control (Lane 2).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 7335 Executive Way | Frederick, Maryland 21704 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0004155

Revision	Date	Description
A.0	19 December 2022	New document for ApoTarget™ Quick Apoptotic DNA Ladder Detection Kit.