Apoptotic DNA Ladder Detection Kit I

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K120
Description	Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. PromoKine's Apoptotic DNA Ladder Detection Kit I 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 electrophoresis. The new procedure increases recovery of small fragmented DNA, and therefore improves the sensitivity of the assay.
Quantity	50 assays
Kit Components	see table below

Component	Quantity	Color code/Cap color
TE Lysis Buffer	1.8 ml	Purple
Enzyme A Solution	0.25 ml	Blue
Enzyme B (Lyophilized)	1 vial	Red
Ammonium Acetate Solution	0.25 ml	Yellow
DNA Suspension Buffer	2 ml	Green

Applications / Assay Protocol

Dissolve Enzyme B with 275 µl ddH_2O and mix well before use. The Enzyme B solution should be refrozen at –70°C immediately after each use or aliquoted and stored at –70°C for future use.

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. Note: For adherent cells, gently trypsinize cells and then pellet cells.
3. Wash cells with PBS (not provided) and pellet cells by centrifugation for 5 minutes at 500 x g. Carefully remove supernatant using pipette.
4. Lyse cells with 35 µl TE Lysis Buffer, gentle pipitting.
5. Add 5 µl Enzyme A Solution, mix by gentle vortexing and incubate at 37°C for 10 minutes. Note: If cells contain high levels of DNase, the incubation step should be skipped, as high DNase levels can digest the DNA ladder, thus generating a smear pattern.
6. Add 5 µl Enzyme B Solution into each sample and incubate at 50°C for 30 minutes or longer (overnight is ok).
7. 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.
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 µl DNA Suspension Buffer.
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) has run to the edge of the gel.
13. Ethidium bromide-stained DNA can be visualized using trans-illumination with UV light and photographed.

Storage & Stability

Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES.