Apoptotic DNA Ladder Detection Kit II

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K170
Description	Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. PromoKine's Apoptotic DNA Ladder Detection Kit II provides an easy and sensitive means for detecting DNA fragmentation in apoptotic cells. The new procedure selectively isolates DNA ladders without interference of regular genomic DNA, which significantly improves detection sensitivity. The kit can detect apoptotic DNA ladders from both tissues and cells with as little as 5% or less apoptotic cell population. The extracted apoptotic DNA ladder fragments can be easily visualized by agarose gel electrophoreses.
Quantity	50 tests

Kit Components

Components	Quantity	Color code/Cap color
DNA Ladder Extraction Buffer	12.5 ml	WM
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

A. Reagent Preparation:
Dissolve Enzyme B with 275 µl ddH$_2$O 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.

B. DNA Ladder Detection 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 minutes at 500 x g. Carefully remove supernatant using a pipette.
For adherent cells, gently trypsinize and then pellet cells. For tissue samples, cut 50 mg tissues into very fine pieces or homogenize tissues in PBS to generate cell suspension (Note: Do not sonicate). Centrifuge to collect cell pellet.
Note: The kit can detect DNA ladders 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 minutes at 1,600 x g (~4, 500 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 minutes. (Note: If cells contain high levels of DNase, then 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 100 µl isopropanol (not provided), mix well, and keep at –20°C for 10 minutes.
8. Centrifuge the sample at maximum speed (≈ 16K x g) for 10 minutes to precipitate DNA. (Note: Microcentrifuges typically generate ≈ 16K x g at 13K x rpm)
9. Remove supernatant, wash the DNA pellet with 0.5 ml 70% ethanol, centrifuge again 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 needed for loading to 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 using trans-illumination with UV light and photographed.

Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

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