Apoptotic DNA Ladder Detection Kit I plus

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K130
Description	Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells. PromoKine's Apoptotic DNA Ladder Detection Kit I plus 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 stained with a highly sensitive dye. The new procedure provides higher sensitivity in comparison to other similar kits on the market. The assay can be used to detect apoptotic DNA ladders in both tissues and cells.
Quantity	50 tests

Kit Components

Components	Quantity	Color code/Cap color
TE Lysis Buffer	1.8 ml	Purple
Enzyme B (Lyophilized)	1 vial	Red
Ammonium Acetate Solution	0.25 ml	Yellow
DNA Suspension Buffer	2 ml	Green
Staining Dye (10,000X)	50 µl	Blue

Applications / Assay Protocol

A. Reagent Preparation:
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.
Staining Dye (10,000X) 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. DNA Ladder Detection 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 minutes at 500 x g. Carefully remove supernatant using pipette.
4. Lyse cells with 35 µl TE Lysis Buffer (gentle pipitting), 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 DNA ladders, thus generating a smear pattern.
5. Add 5 µl Enzyme B Solution into each sample and incubate at 50°C for 30 minutes 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) has run to the edge of the gel.
12. Stain the gel with 50 ml 1X Staining Buffer (1:10,000 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 at short UV wavelength (254 nm) and photographed with a camera equipped with a 520 nm filter.
Note: The illumination time should be no more than 1 minute. Longer illumination may significantly decrease the signal.

Storage & Stability

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

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01/2021