APExBIO
An Apoptosis and Epigenetics Company

Product Information

Quick Apoptotic DNA Ladder Detection Kit

I. Kit Contents:

Component	K2194-50 50 assays	Cap Color	Part Number
TE Lysis Buffer	1.8 ml	Purple	K2194-C-1
Enzyme A Solution	0.25 ml	Blue	K2194-C-2
Enzyme B (Lyophilized)	1 vial	Red	K2194-C-3
Ammonium Acetate Solution	0.25 ml	Yellow	K2194-C-4
DNA Suspension Buffer	2 ml	Greenl	K2194-C-5

II. Introduction:

Internucleosomal DNA fragmentation is a mark of apoptosis in mammalian cells. DNA fragmentation in apoptotic cells can be detected easily and sensitively by the Quick Apoptotic DNA Ladder Detection Kit. The new detection method needs less than 90 minutes to prepare DNA without extraction and columns. Using agarose gel electrophoreses can easily visualize DNA fragmentation. The recovery of small fragmented DNA increases in the new procedure, and the sensitivity of the assay also improves.

III. Reagent Preparation:

Dissolve Enzyme B with 275 µl ddH$_2$O and mix well before use. The Enzyme B solution should refreeze at -70°C immediately after each use, or aliquot and then stored at -70°C for future use.

IV. 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.
   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 min at 500 xg. Carefully remove supernatant using pipette.
4. Lyse cells with 35 µl TE Lysis Buffer, gentle pepitting.
5. Add 5 µl Enzyme A Solution, mix by gentle vortex and incubate at 37°C for 10 min. Note: If cells contain high level of DNase, then the incubation step should be skipped, as high level Dnase can digest DNA ladder generating smear pattern.
6. Add 5 µl Enzyme B Solution into each sample and incubate at 50°C for 30 min 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) run to the edge of the gel.
13. Ethidium bromide-stained DNA can be visualized by trans-illumination with uv light and photographed.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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