Product Information

Enhanced Apoptotic DNA Ladder Detection Kit

I. Kit Contents:

Component	K2202-50 50 assays	Cap Color	Part Number
TE Lysis Buffer	1.8 ml	Purple	K2202-C-1
Enzyme B (Lyophilized)	0.25 ml	Red	K2202-C-2
Ammonium Acetate Solution	1 vial	Yellow	K2202-C-3
DNA Suspension Buffer	0.25 ml	Green	K2202-C-4
Staining Dye (10000X)	50 µl	Blue	K2202-C-5

II. Introduction:

Internucleosomal DNA fragmentation is a mark of apoptosis in mammalian cells. The Enhanced Apoptotic DNA Ladder Detection Kit provides a sensitive and easy means for detecting DNA fragmentation in apoptotic cells. Other commercially available kits require 1 - 2 days to perform the procedure, but the new detection method needs less than 90 minutes to prepare DNA without extraction or using columns. Using agarose gel electrophoreses stained with a highly sensitive dye can easily visualize DNA fragmentation. The new procedure provides higher sensitivity in comparison to other similar kits in the market. The assay can be used to detect apoptotic DNA ladder in both tissues and cells.

III. Reagent Preparation:

Dissolve Enzyme B with 275 µl ddH2O and mix well before use. The Enzyme B solution should refreeze at -70℃ immediately after each use, or aliquot and then stored at -70℃ for future use. Staining Dye (10000X) 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).

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.
3. Wash cells with PBS (not provided) and pellet cells by centrifugation for 5 min at 500 x g. Carefully remove supernatant using pipette.
4. Lyse cells with 35 µl TE Lysis Buffer, gentle pepitting, incubate at 37℃ for 10 min. Note: If cells contain high level of DNase, the incubation step should be skipped, as high level DNase can digest DNA ladder generating smear pattern.
5. Add 5 µl Enzyme B Solution into each sample and incubate at 50℃ for 30 min 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℃ 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) run to the edge of the gel.
12. Stain the gel with 50 ml 1X Staining Buffer (1:10000 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 of short UV wavelength (254 nm) and photographed with camera equipped with 520 nm filter.

Note: The illumination time should be no more than 1 minute. Longer illumination may significantly decrease the signal.

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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