G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Apoptotic-LADDER™ Kit

For Preparing Apoptotic Nucleosomal DNA Ladder

(Cat. # 786-209)

INTRODUCTION

The Apoptotic-LADDER™ Kit contains all of the reagents necessary for the preparation of cellular fractions for the production of apoptosis nucleosomal DNA ladders. The kit does not require the use of toxic phenol. The protocol involves cell lysis; removal of cellular debris and subsequent precipitation of nucleosomal DNA. Nucleosomal DNA is detected after standard 1.8% agarose gel electrophoresis. The protocol provides the choice of preparing nucleosomal DNA ladder either with or without genomic DNA. The kit is suitable for preparing up to 100 apoptotic ladders.

ITEM(S) SUPPLIED (Cat. # 786-209)

Description	Size
Nucleosomal Buffer	30ml
DNA Stripping Solution	10ml
Precipitation Solution	30ml
Longlife™ Proteinase K [5mg/ml]	2 x 0.5ml
TE Buffer	1.5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store the Longlife™ Proteinase K at -20°C and rest of the items at 4°C. When used and stored properly, the kit components are good for 1 year.

ADDITIONAL ITEM(S) REQUIRED

- PBS
- Ethanol
- Microfuge tubes
- Centrifuge
- Agarose gels.

PREPARATION BEFORE USE

DNA Stripping Solution may develop precipitate during storage, allow the solution to warm to room temperature or until precipitate dissolves.

PROTOCOL

A. For Preparing Nucleosomal DNA Ladder with genomic DNA

1. Harvest the cell sample and wash with PBS.
2. Centrifuge to pellet the cells. Resuspend the cells in PBS at approximately 1-2 x 10^6 cells/ml and dispense 1ml into a 1.5 microfuge tube.
3. Pellet the cells by brief centrifugation e.g. 10-15 seconds pulse.
4. Remove and discard the supernatant.
5. Add 150µl of Nucleosomal Buffer to the pellet. Suspend the cells by pipetting up and down a few times.
6. Add 50µl DNA Stripping Buffer and 10µl Longlife™ Proteinase K. Invert the tube a few times to mix the solution.
7. Incubate at 55°C for 90 minutes. Allow the tube to cool to room temperature.
8. Add 150µl of Precipitation Buffer and invert the tube a few times to mix.
9. Centrifuge at 15,000x g for 10 minutes and transfer the supernatant to a clean tube.
10. Add 500µl of ethanol and invert the tube a few times to mix.
11. Incubate at -20°C for 1 hour.
12. Centrifuge at 15,000x g for 10 minutes to recover the nucleosomal DNA precipitate. Remove and discard the supernatant. Centrifuge again for 10 seconds and remove and discard any remaining supernatant.
13. Allow the pellet to evaporate and dry by a brief incubation at 35°C-40°C.
14. Re-suspend the pellet in 25-30µl of TE buffer and incubate at 35°C C for 10-15 minutes or until the DNA pellet is fully hydrated.
15. The sample is now ready to be loaded on 1.8% agarose gel for analysis. During the 1.8% agarose electrophoresis, genomic DNA will be separated from the Nucleosomal DNA fragments revealing the Apoptotic DNA ladder.

B. For Preparing Nucleosomal DNA Ladder Substantially Free From Genomic DNA:

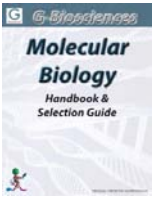
1. Follow the steps 1-4 of protocol A to get clean cell pellet.
2. Add 150µl of Nucleosomal Buffer to the pellet. Suspend the cells by pipetting up and down a few times.
3. Centrifuge at 15,000x g for 5 minutes in cold. Transfer the supernatant into a clean tube.
4. Re-extract the nuclear pellet twice by repeating the above steps 2 & 3. After the second and final extraction, retain the pellet as a control or until the results are concluded with satisfaction.
5. Pool the collected supernatant into a single tube and add 30µl of DNA Stripping solution. Mix the provided Proteinase K solution by tapping the tubes and add 10µl to the sample solution. Invert the tube a few times to mix the solution. Follow the steps 7-14 of protocol A.
6. The sample is now ready to be loaded on the 1.8% agarose gel for analysis.

C. Apoptotic Ladder from Solid Tissue:

Grind 2-5mg tissues in 300µl Nucleosomal Buffer. Add 30µl DNA Stripping Buffer and 10µl Proteinase K solution. Mix and incubate at 55°C for 3-4 hours. Allow the tube to cool to room temperature. Follow the steps 8-14 of protocol A. After Step 14, the sample is ready to be loaded on the 1.8% agarose gel for analysis.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



http://info.gbiosciences.com/complete-molecular-biology-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 11/12/2012 CMH

This page is intentionally left blank

This page is intentionally left blank

www.GBiosciences.com