Cat. # MK600

For Research Use

TaKaRa

ApopLadder Ex™

Product Manual

v201608Da

Table of Contents

I. Description

Apoptosis is characterized by cleavage of chromatin DNA into fragments that are approximately 185 bp (nucleosomal unit lengths). This DNA fragmentation pattern, or laddering, can be used to detect apoptosis.
The ApopLadder Ex Kit selectively extracts the small, fragmented DNA from apoptotic cells. After isolation, the fragmented DNA can be visualized by gel electrophoresis and can be quantified by fluorescent staining.
The ApopLadder Ex Kit contains the necessary reagents for cell lysis, enzymatic degradation of protein and RNA, precipitation of the DNA fragments, and a 6X loading dye for gel electrophoresis.

II. Principle

With this kit, DNA fragments are separated from intact chromatin DNA by treatment of cells with Lysis buffer, centrifugation, and recovery of the supernatant. After enzymatic degradation of impurities (e.g., proteins, RNAs), the DNA fragments are recovered by ethanol precipitation.

III. Features

1. Ready to use
2. High sensitivity : Allows sensitive detection of apoptotic DNAs.
3. Specific : Allows selective extraction of DNA fragments with minimum contamination of intact chromatin.
4. Fast : 2.5 hours to finish all procedures.
5. Quantitative:
   In combination with a fluorescent dye (e.g., SYBR® Green I), DNA fragments can be analyzed by a fluorescent image analyzer, or a fluorescent plate reader.
6. Safe: Phenol and chloroform are not included in this kit.

IV. Components (For 24 assays)

(1) Lysis buffer	1.2 ml x 4
(2) 10% SDS solution	0.48 ml
(3) Enzyme A	0.48 ml
(4) Enzyme B	0.48 ml
(5) Precipitant	1.04 ml x 3
(6) 6X Loading buffer	0.48 ml

V. Storage -20℃

VI. Materials Required but not Provided

- Micropipette
- 1.5 ml microtubes
- 37℃ incubator
- 56℃ incubator
- Vortex mixer
- High speed centrifuge
- Low speed centrifuge
- TE buffer
- PBS (−) buffer
- Ethanol
- 80% ethanol

For detection of DNA fragments: gel electrophoresis equipment, fluorescent image analyzer, and fluorescent dye (e.g., SYBR Green I) are required.

VII. Protocol

1. Wash 10^6 - 10^7 of cells＊1 with PBS (−) and suspend the cells in a small amount of PBS (−). Transfer this suspension into 1.5 ml microtube. Centrifuge at 1,600 X *g* for 5 min at room temperature.
2. Discard the supernatant and loosen a pellet by tapping the bottom of the tube. Add 100 μl of Lysis buffer and vortex for 10 sec.
3. Centrifuge at 1,100 X *g* for 5 min at room temperature. Transfer the supernatant to a new 1.5 ml microtube.
4. Loosen the pellet by tapping the bottom of the tube, add 100 μl of Lysis buffer, and vortex for 10 sec.
5. Centrifuge at 1,100 X *g* for 5 min at room temperature. Transfer the supernatant to the microtube from step 3. The supernatant collected in steps 3 and 5 is the crude extract.
6. Add 20 μl of 10% SDS solution to the crude extract, then add 20 μl of Enzyme A. Incubate at 56℃ for 1 hr.
7. Add 20 μl of Enzyme B to the mixture. Incubate at 37℃ for 1 hr.
8. Add 130 μl of Precipitant, then add 0.95 ml of ethanol. Place at -20℃ for at least 1 hr.
9. Centrifuge it at 12,000 X *g* for 15 min at 4℃. Discard supernatant and wash the pellet with 80% ethanol.
10. Centrifuge again at 12,000 X *g* for 15 min at 4℃. Remove all of the ethanol and dry the pellet.
11. Add 15 - 50 μl of TE buffer＊2 to the tube prepared and dissolve the pellet by mixing well. This solution can be used directly for various downstream analyses (e.g., gel electrophoresis).

＊1 : The amount of fragmented DNA recovered varies depending on the number of apoptotic cells in the population.

＊2 : The amount of DNA fragments recovered varies depending on the efficiency of apoptosis induction. (Refer to Q1 in Q & A). Dissolve the pellet with a small volume of TE buffer initially.

VIII. Applications

[To confirm DNA ladder by gel electrophoresis]

Dilute the sample 5 : 1 with 6X Loading buffer, adjust the total volume to 6 - 15 μl, and load on an agarose gel.

[To perform fluorescence detection using SYBR Green I]

Dilute the DNA fragment sample with TE buffer at an appropriate ratio. Dilute the fluorescent dye 1 : 1000 with TE. Add 5 μl of the diluted dye to 50 μl of diluted sample. Fluorescence can be measured using an appropriate instrument (e.g., fluorescent plate reader, spectrophotofluorometer).

XI. Q & A

Q1 : What kind of samples can be used with ApopLadder Ex?

A1 : This kit is intended for use with cultured cells. However, any uniform cell population can be used. For example, lymphocytes separated from whole blood using BD Vacutainer CPT (Becton Dickinson) or isolated from spleen can be used. This kit cannot be used for direct extraction from tissues.

Q2 : Can DNA fragments be extracted from frozen cells?

A2 : Using frozen cells is not recommended. DNA fragments can be extracted and detected from cells that have been frozen and thawed three times, but recovery of DNA fragments is greatly decreased.

Q3 : How should DNA fragment solutions be stored? How long is the solution stable?

A3 : The DNA fragment solution should be aliquoted and stored at -20℃. Do not freeze and thaw the solution. The solution is stable at least for one month at -20℃ and for one week at 4℃.

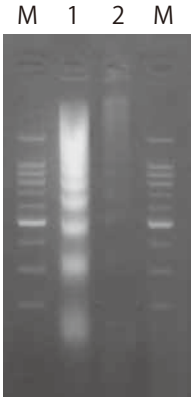
Q4 : What control experiment can be used to check for extraction?

A4 : DNA fragmentation caused by apoptosis is highly affected by following factors: type of target cells, type of inducer, concentration of inducer, and timing of induction. HL60 cells treated with 1 mM staurosporine (Sigma-Aldrich, Code. S4400) can be used as a control experiment.

< Control experiment using HL60 cells >

HL60 cells were seeded at 10^6 cells/ml in a 90 mm dish. Staurosporine was added at a final concentration of 3 μM at the same time. The cells were incubated for 5 hours * (37℃, 5 % CO_2).
The cells were washed with PBS (−) and suspended with a small amount of PBS (−). This suspension (5 x 10^6cells) was transferred to 1.5 ml microtube and then centrifuged at room temperature at 1,600 X *g* for 5 min.
The fragmented DNA was obtained using the ApopLadder Ex Kit. The final DNA pellet was dissolved in 20 μl of TE buffer. 5 μl was used for gel electrophoresis.

* : The effect of staurosporine depends on the condition of the cultured cells. The ladder in Figure 1 was obtained for 3 - 5 hours culture.



M : 100 bp DNA Ladder (200 ng)
1 : staurosporine treated
2 : untreated

Figure 1

Q5 : Why does electrophoresis show a smear instead of a distinct ladder?

A5 : Possible problems:
1) Apoptosis induction was too long and DNA was cut non-specifically.
2) DNase contamination during the extraction procedures.

Solutions:
1-1) Start sampling earlier.
2-1) Avoid DNase contamination (e.g., using gloves).

Q6 : Why does electrophoresis show no bands?

A6 : Possible problems:
1) Apoptosis did not occur.
2) The amount of fragmented DNA recovered was below the limit of detection.

Solutions:
1-1) Increase the amount of apoptosis inducer or increase the induction time.
1-2) Test whether apoptosis occurred using another methods [e.g., *In situ* Apotosis Detection Kit (Cat. #MK500), morphological observation].
2-1) Increase the number of cells treated.
2-2) In step 11, dissolve the DNA obtained after ethanol precipitation in a smaller amount of TE buffer.

Q7 : The SDS precipitated when 10% SDS solution was thawed at room temperature.

A7 : Thaw at 40℃.

Q8 : Besides SYBR Green I, can other fluorescent dyes be used in the assay?

A8 : PicoGreen (Thermo Fisher Scientific) can be used.

Q9 : What kind of agarose gel is suitable for detection of DNA fragments?

A9 : A 3% PrimeGel ™ Agarose PCR-Sieve (Cat. #5810A) gel is recomended.

Q10 : The 6X Loading buffer in this kit turned yellow. Can this buffer still be used?

A10 : Yes. Yellow dye is used in this Kit, but does not affect visualization of DNA bands.

Q11 : When assaying adherent cells, what method should be used to detach the cells.

A11 : We recommend using a cell scraper. Scraping provides better results than trypsin.

X. Related products

500 bp DNA Ladder (Cat. #3411A/B)
100 bp DNA Ladder (Cat. #3407A/B)
In situ Apoptosis Detection Kit (Cat. #MK500)