ApopLadder Ex™ (Cat.# MK600)

Frequently Asked Questions: ApopLadder Ex Kit

Apoptosis is associated with the fragmentation of chromosomal DNA into approximately 180 bp nucleosome fragments. The ApopLadder Ex™ Kit permits the selective extraction of small, fragmented DNA from apoptotic cells while minimizing chromatin contamination. This kit also supports highly sensitive detection and quantitation of fragmented DNA. Detection of fragmented DNA following extraction can be performed via a DNA fragmentation assay involving gel electrophoresis and visualization of DNA fragments characteristic of cells undergoing apoptosis. The ApopLadder Ex Kit is optimized for sensitive, specific and rapid detection of fragmented DNA.

Answers to frequently asked questions about ApopLadder Ex are presented here. For additional information, refer to the product User Manual and web page.

Q1: What is the typical quantity and quality of the DNA fragments obtained using the ApopLadder Ex Kit?

A1: The quantity and quality varies depending on the cell type, method of apoptosis induction, and time of sampling. The following data are an example of typical results:

Cell type	Induction of apoptosis	Amount of sample	DNA yield	OD_{260} / OD_{280}
P3U1	Actinomycin D (10 µM), 20 hours	1×10^6 cells	> 20 µg	> 1.9
HL60	Staurosporine (1 µM), 5 hours	1×10^6 cells	> 25 µg	> 1.9

Q2: What kind of samples can be used with ApopLadder Ex?

A2: The 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.

Q3: Can DNA fragments be extracted from frozen cells?

A3: Using frozen cells is not recommended. DNA fragments can be extracted and detected from cells that have been frozen and thawed three times, but the recovery of DNA fragments is greatly decreased.

Q4: How should isolated DNA fragments solution be stored? How stable is the solution?

A4: The DNA fragment solution should be aliquoted and stored at -20°C. Do not freeze and thaw the solution. The solution is stable at least for one month at -20°C and for one week at 4°C.

Q5: What positive control experiment is recommended to check for successful extraction?

A5: DNA fragmentation caused by apoptosis is highly affected by following factors: cell type, type of apoptosis inducer, concentration of inducer, and induction time. HL60 cells treated with 1 mM staurosporine can be used as a control experiment.

Q6: A smear instead of a distinct ladder is observed upon gel electrophoresis. Why might this occur?

A6: Possible causes:

1) Apoptosis was induced for too long, and the DNA was cut non-specifically.

2) DNase contamination during the extraction procedures.

- Continued -

Possible solutions:

1) Start sampling at earlier time points.

2) Avoid DNase contamination (e.g., use gloves).

Q7: No bands are observed upon gel electrophoresis. Why might this occur?

A7: Possible problems:

1) Apoptosis did not occur.

2) The amount of fragmented DNA recovered was below the limit of detection.

Possible solutions:

1-1) Increase the amount of apoptosis inducer or increase the induction time.

1-2) Test whether apoptosis occurred using another method (e.g., In situ Apotosis Detection Kit (Cat.#MK500), morphological observation).

2-1) Increase the number of cells treated.

2-2) In step 11 of the protocol for ApopLadder Ex, dissolve the DNA obtained after ethanol precipitation in a smaller amount of TE buffer.

Q8: SDS precipitated when the 10% SDS solution was thawed at room temperature. How can this be avoided?

A8: Thaw at 40°C.

Q9: Besides GelStar and SYBR® Green I, can other fluorescent dyes be used in the assay?

A9: PicoGreen® (Life Technologies) can be used.

Q10: What kind of agarose and what percentage gel is suitable for detection of DNA fragments?

Q10: We recommend a 3% NuSieve® 3:1 Agarose gel.

Q11: The 6X Loading buffer in this kit is yellow. Can this buffer still be used?

A11: Yes. Yellow dye is used in this Kit, but it does not affect visualization of DNA bands.

A12: When assaying adherent cells, what method should be used to detach the cells?

A12: We recommend using a cell scraper. Scraping provides better results than trypsin treatment.