ApopLadder Ex™ (Cat.# MK600)

Application: Detection of the Early Stages of Apoptosis Using ApopLadder Ex™

Apoptosis is characterized by fragmentation of chromosomal DNA into multiples of approximately 180 nt, corresponding to nucleosome fragments. This fragmentation process is also referred to as DNA laddering. The ApopLadder Ex Kit allows selective extraction of small, fragmented DNA from apoptotic cells while minimizing chromatin contamination. Using a fluorescent nucleic acid dye such as SYBR® Green I, DNA fragments purified using ApopLadder Ex can be quantified, providing a sensitive method of apoptosis analysis.

This application note illustrates the use of ApopLadder Ex and SYBR Green I quantification of DNA fragmentation for the detection of the early stages of apoptosis in mouse and human cell lines.

Methods

P3U1 (murine myeloma cell line) cells were treated for 20 hours with various concentrations of actinomycin D. HL60 (human promyelocytic leukemia cell line) cells ($3x10^5$ cells) were treated with 1 µM staurosporine. Apoptosis was analyzed by trypan blue staining and/or analysis of DNA fragmentation using ApopLadder Ex (Cat. # MK600).

Results

In general, the permeability of the cell membrane increases during late apoptosis, and apoptotic cells can be visualized by trypan blue staining. However, DNA fragmentation occurs early in apoptosis, often before changes in cell membrane permeability occur. We hypothesized that the highly sensitive quantitative detection possible with ApopLadder Ex could be used to detect early stages of apoptosis.

In P3U1 cells, trypan blue staining indicated an increase in apoptosis when cells were treated with 30 ng/ml actinomycin D (Figure 1). In contrast, in these same cells, an increase in apoptosis (as indicated by an increase in fluorescence intensity) was detected with treatment of as little as 5 ng/ml actinomycin D when fragmented DNA was extracted with ApopLadder Ex and quantified with SYBR Green I.

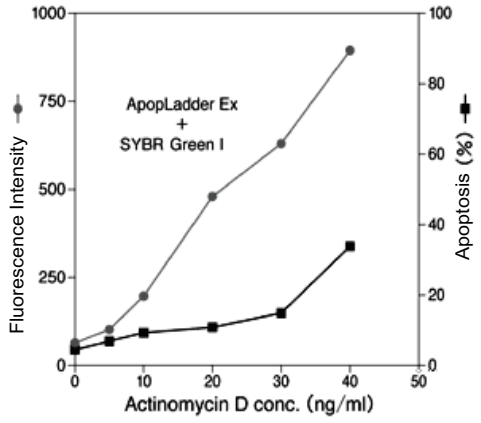

Figure 1. Assessing apoptosis in P3U1 cells with trypan blue or ApopLadder Ex and SYBR Green I. Cells were treated with various concentrations of actinomycin D (x-axis) for 20 hours. Apoptosis was measured (y-axis) using either trypan blue staining (percentage of positive cells plotted on the y-axis) or ApopLadder Ex Kit in combination with SYBR Green I (fluorescence intensity).

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To further assess the ability of the ApopLadder Ex to detect early stages of apoptosis, DNA laddering was analyzed over time in HL60 cells treated with staurosporine. Using gel electrophoresis to analyze DNA isolated with ApopLadder Ex, DNA fragmentation was clearly detected after 3 hours of treatment (Figure 2a). Using SYBR Green I to quantify the isolated fragmented DNA indicated an increase in DNA fragmentation apoptosis after just 1 hour of staurosporine treatment (Figure 2b). In contrast, no morphological changes of any kind were observed with an optical microscope after exposure with staurosporine from 1 to3 hours (data not shown).

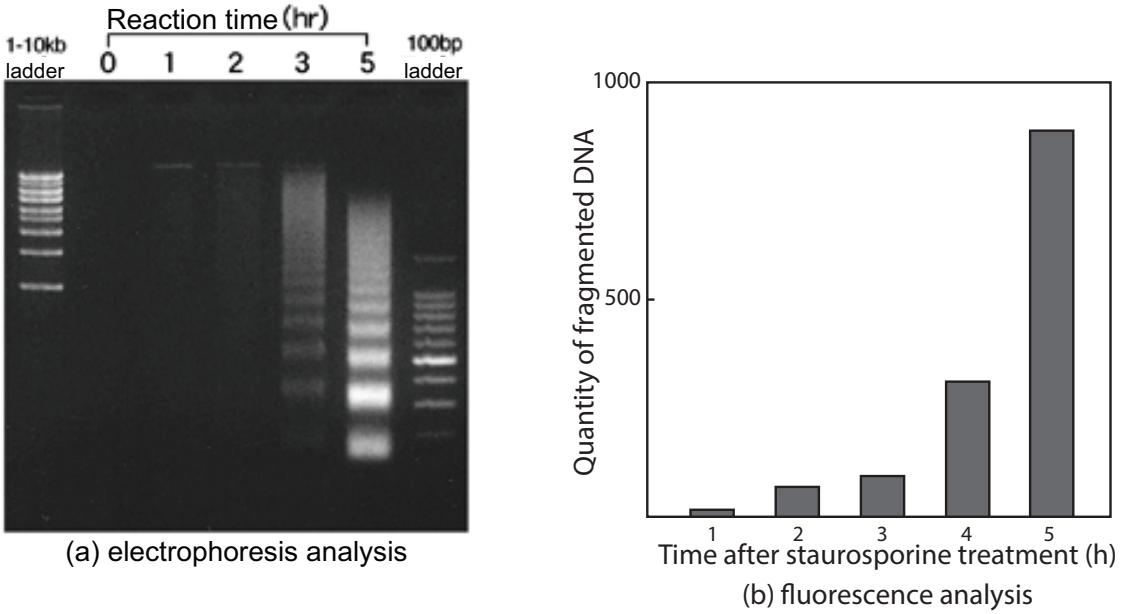

Figure 2. Time course analysis of apoptotic DNA fragmentation in HL60 cells treated with staurosporine. At various time points (0, 1, 2, 3, or 5 hours), fragmented DNA was isolated using the ApopLadder Ex Kit. The isolated DNA was analyzed by gel electrophoresis on a 2% agarose gel (a) or quantified using SYBR Green I (b).

Conclusion

Quantification of fragmented DNA isolated using the ApopLadder Ex Kit is an effective method for sensitive detection of the early stages of apoptosis.