ApopLadder Ex™ (Apoptotic DNA Fragment Extraction Kit (Cat.# MK600))

Application: Apoptosis-induced DNA Fragmentation Detection using the ApopLadder Ex™ Kit

Apoptosis is a programmed cell death process during which chromosomal DNA is systematically degraded into approximately 180 base pair fragments and multiples thereof. The accumulation of these fragments in apoptotic cells is referred to as "DNA laddering". Analysis of apoptosis is facilitated by the ApopLadder Ex Kit, which allows selective extraction of small, fragmented DNA from apoptotic cells while minimizing chromatin contamination.

This application note shows the use of the ApopLadder Ex Kit for isolating fragmented DNA from mouse myeloma cells treated with actinomycin D, a cytotoxic agent that induces apoptosis.

Methods

Fragmented DNA was isolated from P3U1 cells using the ApopLadder Ex (Cat.# MK600). The cells had been treated with actinomycin D (10 µM) for 20 hours. DNA fragments were resolved on a 1% agarose gel and visualized using ethidium bromide.

Results

As shown in Figure 1, a smear of high molecular weight DNA corresponding to intact chromatin was recovered in the pelleted fraction (ppt), whereas little high molecular weight DNA was detected in the selectively extracted supernatant (sup) fraction. This result indicates that fragmented DNA was efficiently extracted using this kit. Fragmented DNA ladders were detected only from P3U1 cell samples that were treated with actinomycin D.

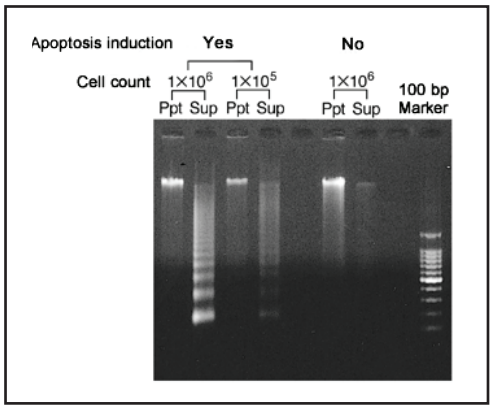

Figure 1. Visualization of DNA fragmentation induced by Actinomycin D.

Conclusions

Cells treated with actinomycin D, an inducer of apoptosis, displayed significant DNA fragmentation as determined by DNA isolation with ApopLadder Ex Kit and analysis by gel electrophoresis. In the control cells (not treated with actinomycin D), fragmented DNA was not detected, consistent with the observation of few apoptotic cells in this sample by the TUNEL method (*In situ* Apoptosis Detection Kit [Cat.# MK500]). Using the ApopLadder Ex Kit, detection of apoptosis could be achieved with as few as 1×10^5 cells when the estimated apoptosis rate was approximately 50%.