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A rapid and simple method for the isolation of apoptotic DNA fragments

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During development different cells of multicellular organisms die by a physiological process referred to as programmed cell death or apoptosis (1). In difference to necrosis the apoptotic death requires *de novo* protein synthesis (2) and is therefore often called cellular suicide. Besides cell morphology fragmentation of chromatin into units of single or multiple nucleosomes is specific for apoptosis and one of the easiest ways to distinguish programmed cell death and toxic necrosis.

Besides more sophisticated techniques like cytofluorometry after staining of cells with propidium iodide (3) and *in situ* labelling with terminal nucleotidyltransferase (4) the appearance of the nucleosomal DNA ladder in agarose gels has become the hallmark of programmed cell death. The isolation of apoptotic DNA is performed either with isolated nuclei (3,5) or with total cells (4,6). In most protocols intact chromatin compromises handling of the samples, especially if the proportion of apoptotic cells is low.

Here we describe a simple and reproducible technique for the isolation of apoptotic DNA fragments. The method works without time consuming organic extractions and is suitable for the management of a high number of samples. After harvesting the cell samples are washed with phosphate buffered saline and pelleted by centrifugation. The cell pellets are then treated for 10 s with lysis buffer (1% NP-40 in 20 mM EDTA, 50 mM Tris-HCl, pH 7.5; 10 μl per 10^6 cells, minimum 50 μl). After centrifugation for 5 min at 1600×g the supernatant is collected and the extraction is repeated with the same amount of lysis buffer.

The supernatants (and the resuspended nuclei as control for the complete recovery of the apoptotic DNA fragments) are brought to 1% SDS and treated for 2 h with RNase A (final concentration 5 μg/μl) at 56°C followed by digestion with proteinase K (final concentration 2.5 μg/μl) for at least 2 h at 37°C. After addition of 1/2 vol. 10 M ammonium acetate the DNA is precipitated with 2.5 vol. ethanol, dissolved in gel loading buffer, and separated by electrophoresis in 1% agarose gels. Otherwise it could be used for molecular analysis to purpose.

Figure 1 compares the NP-40 lysis method with a frequently used method for isolation of apoptotic DNA (7). It shows, that the yield of apoptotic DNA is increased in the NP-40 lysis method compared to the phenol extraction procedure. Furthermore the smear of genomic DNA, which blurs the conventional apoptotic ladder is strongly reduced. The comparison of the NP-40 lysates

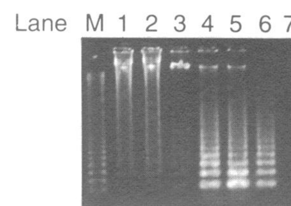

Figure 1. Comparison of two methods for the preparation of apoptotic DNA fragments. Apoptosis was induced in CTLL cells by Il-2 deprivation for 0, 3, and 14 hours, respectively. The viability was estimated by propidium iodide staining (3). Lane M: 142 bp ladder; PBMC: freshly prepared peripheral blood mononuclear cells from healthy donors. NI: NP-40 lysis; St: standard procedure (6).

lane	1	2	3	4	5	6	7
cell type	CTLL	CTLL	CTLL	CTLL	CTLL	CTLL	PBMC
cell count	10^6	10^6	10^5	10^6	10^6	10^5	10^8
Il-2 deprivation	0 h	3 h	14 h	0 h	3 h	14 h	n.d.
viability	96%	96%	45%	96%	96%	45%	99%
method	St	St	St	Nl	Nl	Nl	Nl

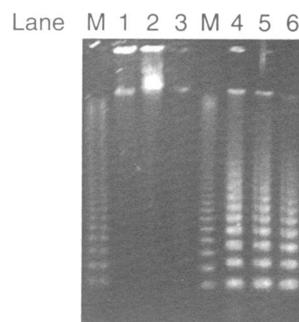

Figure 2. Apoptotic DNA fragments prepared by NP-40 lysis of CTLL. Apoptosis was induced in CTLL cells by Il-2 deprivation for 0, 3, and 14 hours, respectively. Lane M: 142 bp ladder; SN: supernatant of NP-40 lysates; P: Pellet of NP-40 lysates.

lane	1	2	3	4	5	6
cell count	10^6	10^6	10^5	10^6	10^6	10^5
IL-2 deprivation	0 h	3 h	14 h	0 h	3 h	14 h
viability	96%	96%	45%	96%	96%	45%
fraction	P	P	P	SN	SN	SN

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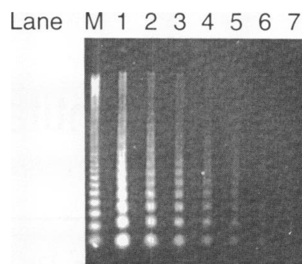

Figure 3. Apoptotic DNA fragments prepared by NP-40 lysis of CTLL. Cells were made apoptotic by Il-2 deprivation for 14 hours, respectively. The percentage of apoptotic cells was 55% (as estimated by propidium iodide staining (3)). Lane M: 142 bp ladder;

lane	1	2	3	4	5	6	7
total cell count/10^3	100	50	25	13	6.3	3.1	1.6
apoptotic cell count/10^3	55	28	14	6.9	3.4	1.7	0.9

with the corresponding nuclear pellets reveals that virtually all the apoptotic DNA can be recovered from the supernatants, whereas intact chromatin is found in the pellet (Figure 2). We conclude that nuclei of apoptotic cells were lysed by the NP-40 buffer and that apoptotic DNA fragments were released into the supernatant, whereas nuclei of living cells remained intact and were therefore pelleted in the low speed centrifugation step. Nucleosome ladder formation due to artificial degradation during the isolation procedure could be excluded since no apoptotic DNA fragments could be observed in freshly isolated PBMC even if 10^7 cells were processed (Figure 1). The detection limit of DNA fragments using ethidium bromide staining is about 1700 apoptotic CTLL, if a gel with narrow wells (1 mm) is used for agarose electrophoresis (Figure 3).

Taken together the main advantages of this method are: 1) The preparation is performed in a single tube after cell lysis. 2) It is less time consuming and expensive as compared to conventional methods. 3) The method removes intact chromatin in a simple centrifugation step and is therefore suitable for the detection of low amounts (less than 1%) of apoptotic cells in the presence of a large number of living cells (data not shown).

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