Apoptosis Assay Methods
Methods for studying apoptosis in cell populations

A

A

2.1.3 Summary of methods for studying apoptosis in cell populations

Method/Roche Applied Science product	Parameter analyzed	Label	Assay principle	Advantages	Limitations	For product information, see
DNA Fragmentation Assay, radioactive[11, 12]	DNA fragmentation (LMW and HMW-DNA)	$[^3H]$-TdR or $[^{125}I]$- UdR, prelabel	● DNA fragments are released from the cytoplasm of apoptotic cells after lysis with non-ionic detergent. ● The LMW DNA is separated from nuclear HMW DNA by centrifugation. ● The radioactivity in the supernatant and in the pellet is determined by LSC.	● Quantitative measurement over a large range (several orders of magnitude) ● Sensitive (10^3–10^4 cells/test required) ● Suitable for analysis of cell-mediated (cytotoxicity) effects	● Radioactive isotope ● Requires prelabeling and extensive washing of the target cells ● Limited to target cells proliferating *in vitro* ● Increased background due to free $[^3H]$-TdR in the cytoplasm	
DNA Fragmentation Assay, non-radioactive[13] **Cellular DNA Fragmentation ELISA**	DNA fragmentation (LMW DNA)	BrdU, prelabel	● DNA fragments are released from the cytoplasm of apoptotic cells after lysis with a non-ionic detergent. ● The LMW DNA is separated from nuclear HMW DNA by centrifugation. ● The supernatant is analyzed by ELISA.	● Sensitive (10^3–10^4 cells/test required) ● Labeled cells do not have to be washed ● Optimal for microtiter plate format ● Non-radioactive ● Suitable for analysis of cell-mediated (cytotoxicity) effects	● Prelabeling of the target cells required ● Can only assay target cells proliferating *in vitro* ● Narrow range of quantitative measurement (only one order of magnitude)	page 64 of this guide
JAM Test[14]	DNA fragmentation (HMW DNA)	$[^3H]$-TdR, prelabel	● Cells are harvested by vacuum aspiration onto glass fiber filters. While LMW-DNA is washed through the filters, the HMW DNA is retained on these filters. ● The radioactivity retained on the filters is measured by LSC.	● Sensitive (10^3–10^4 cells/test required) ● Only 1 washing step required for the labeled cells ● Low spontaneous release: cytotoxic events causing low cell lysis over prolonged period of time (8–24 h) can be studied ● Optimal for microtiter plate format	● Radioactive isotope ● Prelabeling of the target cells required ● Limited to target cells proliferating *in vitro* ● In apoptotic cells, DNA is only partially lost: viable and damaged cells are separated by only a narrow range of assay values	
Alkaline Elution Analysis[15]	DNA fragmentation (LMW and HMW-DNA)	$[^3H]$-TdR, prelabel	● Cells are loaded onto polycarbonate filters. ● The filters are incubated with three different buffer solutions containing SDS, pH 10, SDS + Proteinase K, pH 7, or SDS, pH 12.3. ● The radioactivity in each fraction (LMW DNA) as well as the radioactivity retained on the filter (HMW DNA) is quantified by LSC.	● Differential elution allows the detection of strand breaks in DNA, DNA-interstrand crosslinks and DNA-protein crosslinks	● Radioactive isotope ● Prelabeling and washing of the target cells required ● Limited to target cells proliferating *in vitro* ● Insensitive (10^6 cells/test required) ● Labor-intensive and time-consuming: only a few tests may be performed simultaneously	
DNA Ladder Assay[16] **(LMW and HMW DNA by size)** **Apoptotic DNA Ladder Kit**	DNA fragmentation	none	● Cellular DNA is isolated by extraction and quickly purified. ● Purified total DNA (LMW and HMW DNA) is analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide.	● Hallmark of apoptosis: demonstration of the mono- and oligonucleosomal DNA fragments (180 bp multimers) ● No prelabeling of the cells required: not limited to cells which proliferate *in vitro* ● Non-radioactive	● No quantitative measurement ● Insensitive: More than 10^6 cells/test required ● Labor-intensive and time-consuming: only a few tests may be performed simultaneously	page 13 of this guide
Nucleosome Quantification ELISA[13] **Cell Death Detection ELISA^PLUS^**	DNA fragmentation (LMW DNA in association with histones)	none	● Histone complexed DNA-fragments (mono- and oligonucleosomes, LMW DNA) are released from the cytoplasm of apoptotic cells after lysis. ● The LMW DNA is separated from nuclear HMW DNA by centrifugation. ● The supernatant is analyzed by ELISA.	● Sensitive (10^2–10^4 cells/test required) ● No prelabeling of the cells required: not restricted to cells which proliferate *in vitro* ● Non-radioactive ● Detection of DNA and histones in one immunoassay demonstrates mono- and oligonucleosomal DNA fragments	● Samples have to be analyzed immediately because storage reduces ELISA signal ● Not recommended for tissue homogenates. Increased background could occur due to activation of nucleases during sample preparation.	page 15 of this guide
DNA Ladder Assay, radioactive [17]	DNA fragmentation (LMW and HMW by size)	γ-$[^{32}P]$-ATP, postlabel	● Cellular DNA is isolated by extraction and quickly purified. ● Purified total DNA (LMW and HMW DNA) is labeled at the 5'end with γ-$[^{32}P]$-ATP by T4 Polynucleotide Kinase. ● $[^{32}P]$-labeled DNA is separated by agarose gel electrophoresis and quantitated in the dried gel by a blot analyzer.	● Definitive marker of apoptosis: demonstration of the mono- and oligonucleosomal DNA fragments (180 bp multimers) ● No prelabeling of the cells required: not limited to cells which proliferate *in vitro* ● Highly sensitive (1000 x more sensitive than ethidium bromide): allows earlier detection of DNA fragmentation after induction of apoptosis	● Labor-intensive and time-consuming: only a few tests may be performed simultaneously ● Radioactive assay (^{32}P) ● End-labeling of purified DNA required	
Protease Activity Assay **Caspase 3 Acitivity Assay**	Activation of caspases (Caspase 3)	none	● Apoptotic process including activation of the caspase cascade is induced in cells by desired method. ● Cells are lysed and cell extracts are prepared. ● Activated caspase 3 is captured out of cellular lysates by an Anti-caspase 3 antibody ● Quantification of fluorochromes cleaved from a caspase specific substrate.	● Quantitative assay, cleavage of substrate is proportional to concentration of activated caspase 3 in samples ● Detection of very early stages of apoptosis ● Highly specific for caspase 3, no cross reactions with other members of the caspase family	● High cell numbers needed ● Fluorescence reader, equipped with special fluorescence filters needed	page 22 of this guide
Protease Activity Assay **Anti-PARP**	Discrete cleavage of DNA repair enzyme (PARP)	none	● Cells are treated with an apoptosis-inducing agent, which leads to induction of caspase 3 and the cleavage of Poly-ADP-Ribose-Polymerase (PARP). ● Cell extracts are prepared with SDS, fractionated by SDS-PAGE, and transferred to a PVDF membrane by western blotting. ● Blot is probed with an antibody to PARP, then with a peroxidase-labeled secondary antibody. ● Cleavage products of PARP (about 85 kD) on the membrane are revealed after an incubation with a peroxidase substrate.	● Flexible, can be used with many different types of cells ● No prelabeling of cells required: not limited to cells which proliferate *in vitro* ● Non-radioactive ● Marker for very early stage of apoptosis	● Insensitive (requires 10^5–10^6 cells/test) ● Labor-intensive and time-consuming: only a few tests may be performed simultaneously	page 27 of this guide

▲ **Table 4: Methods for studying apoptosis in cell populations.**