New in Apoptosis Imaging: Dual Detection of Self-Execution and Waste-Management

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Abstract

Apoptosis enables the orderly disposal of unwanted cells without an inflammatory response. In the process, DNA fragments with defined ends are created, a phenomenon used by many commercially available apoptosis detection kits. While many kits are very sensitive and versatile, all have a major drawback: they are only able to detect the presence of DNA fragments with DNase I type ends, 3′OH/5′PO_4. However, apoptosis also generates fragments with DNase II type ends, 3′PO_4/5′OH (Figure 1). Here we report a new apoptosis detection method which enables researchers to detect both types of DNA fragments simultaneously in a single sample.

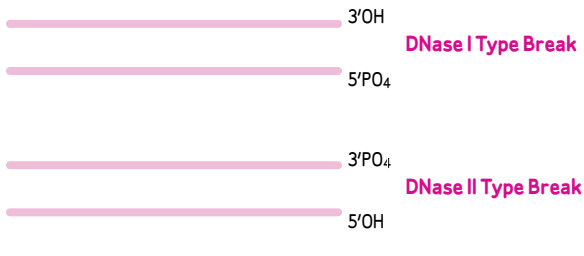


Figure 1. DNase I and II type breaks.

Introduction

During apoptosis, cells undergo sequential steps leading to their complete disassembly and disappearance. Massive and systematic DNA fragmentation is a characteristic feature of this process. Not surprisingly, these DNA fragments are often used as specific markers in apoptosis detection. DNA fragments created in apoptosis are not random. Usually, they possess blunt ends or short, single-nucleotide staggers[1,2]. A double-strand (ds) break forms when a DNA duplex is cut through, exposing the 3′ and 5′ ends of the two DNA strands. These ends can carry either a phosphate (PO_4) group or a hydroxyl (OH) group (Figure 1). The distribution of these groups provides important information about the enzyme that cut the DNA.

Cuts with a 3′OH/5′PO_4 configuration are termed DNase I type cuts. Cuts with the opposite configuration, 3′PO_4/5′OH, are called DNase II type[3,4]. These cuts received their names because they match the cleavage patterns of the two major nucleases, DNase I and DNase II. These names are used for convenience – the actual apoptotic nuclease involved may or may not be related to DNase I and II.

Different properties of DNase I cleavage are used by various apoptosis detection assays. For example, several of Millipore's ApopTag® kits specifically detect one marker of DNase I cleavage by labeling the 3′OH groups with help of

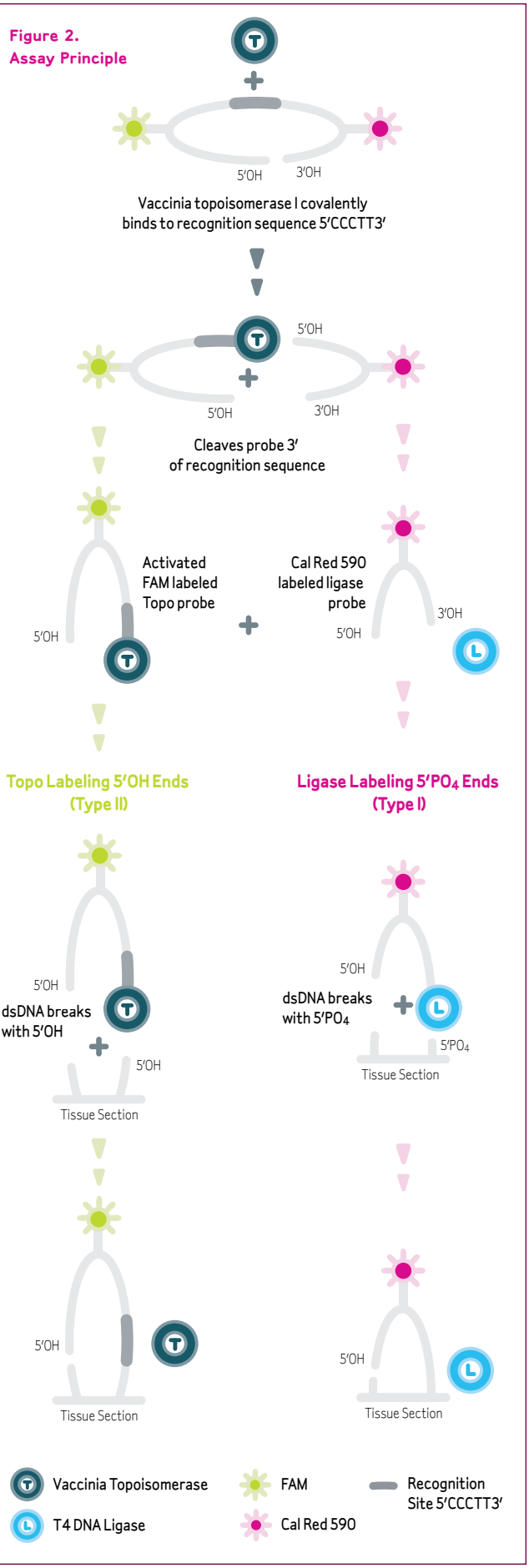
the terminal deoxyribonucleotidyl transferase (TdT) enzyme. Millipore's related assay, the ApopTag Oligo Ligation kit, detects three parameters at once by using T4DNA ligase and is even more specific. Although highly sensitive, both of these assays detect only DNase I type fragments—leaving cells with DNase II type cleavage undetected. Until recently, there were no methods that could specifically detect DNase II type breaks.

New developments in apoptosis research have changed the very concept of apoptotic cell elimination and significantly increased the value of detecting DNase II type breaks. Apoptosis is no longer viewed as an individual cellular event. The complete apoptotic process is now thought to include two phases: self-execution and externally-controlled elimination of apoptotic cell corpses by waste-management cells[7]. The externalized waste-control phase is essential, highly conserved, and considered to be even more important than the internal phase of cell disassembly[5,6,7], because it ensures the complete degradation of the dying cell's DNA. This prevents release of pathological, viral, and tumor DNA and self-immunization.

DNase II plays a fundamental role in the waste-management phase of apoptosis[5,8]. DNase II is present in lysosomes, the sac-like organelles that contain digestive enzymes used in breaking down cellular components. The enzyme destroys the DNA of apoptotic cells after their corpses are engulfed by tissue macrophages and other waste-management cells.

The new ApopTag ISOL Dual Fluorescence Apoptosis Detection kit addresses these important developments[9]. It offers new dual-detection technology which labels both the self-execution and waste-management phases of apoptotic DNA degradation.

Figure 2.
Assay Principle



Methods

This new technique uses a novel oligonucleotide probe for detection[9]. This self-annealing oligo contains two complementary base sequences that spontaneously form a dual hairpin secondary structure (Figure 2). The oligo also has two fluorescent labels (FAM and Cal Red 590) at opposite poles of the dual hairpin.

Topoisomerase I cuts the DNA at the 3′ end of the recognition site, splitting the dual hairpin oligo into two differently-labeled hairpin oligonucleotides. The biochemical specificity of the provided enzymes impacts the detection aspect of the protocol, in that vaccinia topoisomerase I will recognize and ligate the FAM oligo to 5′ OH DNA ends (DNase type II cuts) whereas T4 DNA Ligase will recognize and ligate the CR590 labeled oligo to 5′ PO_4 DNA ends (DNase type I cuts).

The actual staining protocol is quite simple, and works with a variety of sample types including paraffin-embedded tissue, frozen tissue sections, cell suspensions, and adherent cells. Samples are fixed and prepared for staining according to their type, incubated for 10-16 hours with the dual labeling solution, washed, counterstained, and visualized using fluorescence microscopy.

Results & Discussion

Unlike its predecessors, the ApopTag ISOL Dual Fluorescence Apoptosis Detection kit specifically detects both DNase I and II type breaks. Both vaccinia topoisomerase I and T4 DNA ligase labeled only one type of DNA break each when tested in bovine tissue (Figure 3).

Tests in rat thymus showed the full capacity of this new kit. Cortical macrophages with engulfed nuclear material and apoptotic thymocytes were clearly labeled. Cytoplasmic fluorescence revealed lysosomes containing DNA with 5′OH double-strand breaks. Surrounding thymocytes underwent apoptosis and had 5′PO_4 double-strand breaks located at the periphery of their nuclei (Figure 4). Both phases are also seen in the lower magnification view of apoptotic thymus tissue in Figure 5.

Through the use of novel oligonucleotide probes, the ApopTag ISOL Dual Fluorescence Apoptosis Detection kit overcomes the disadvantages of traditional apoptosis detection kits. This new kit labels both DNase I and DNase II-type apoptotic cleavage, expanding the range of detectable DNA breaks and addressing the wider impact of apoptosis in tissue. The dual-detection format enables scientists to clearly visualize both the self-execution and waste-management phases of apoptosis in a single sample, making research faster and more convenient than ever before.

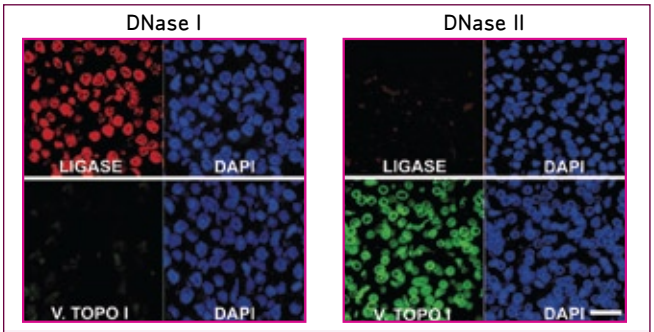


Figure 3. Selective detection of two major types of DNA damage. Sections of normal bovine adrenal tissue were treated with either DNase I to produce 3′OH/5′PO_4 blunt-ended breaks or DNase II to produce 3′ PO_4/5′ OH blunt-ended breaks. Bar - 25 µm.

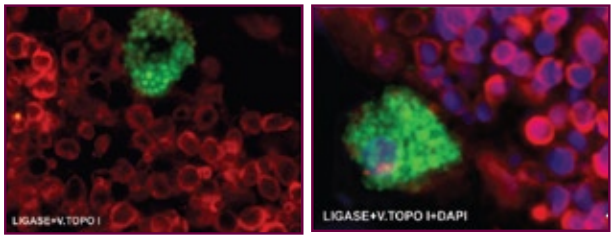


Figure 4. Dual detection of the execution and waste-management phases of apoptosis. Dexamethazone-treated apoptotic rat thymus. Red: ligase-based detection of self-execution phase, green: topoisomerase-based detection of waste-management phase, blue: DAPI nuclear stain. Bar - 15 µm.

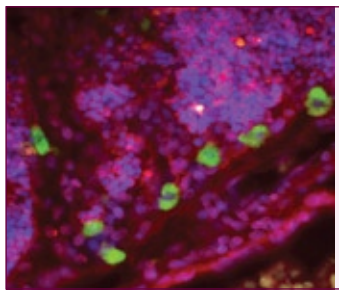


Figure 5. Macrophages loaded with apoptotic DNA in the waste-management phase of apoptosis in thymus. Green: macrophages with engulfed apoptotic corpses (TOPO labeling), red: apoptotic thymocytes (*in situ* ligation), blue: nuclear staining (DAPI).

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Millipore Products

Description	Cat. No.
ApopTag ISOL Dual Fluorescence Apopotosis Detection Kit (DNase Types I & II)	APT1000
ANTI-FAS Monoclonal	05-201
Anti-Caspase 3, active (cleaved) form	AB3623
Anti-Bak, NT	06-536
Anti-Bax, NT	06-499
Anti-Clusterin α chain (human), clone 41D	05-354
Anti-Cyclophilin A	07-313
Anti-Caspase 1	06-503
Anti-DNA, single stranded specific, clone F7-26	MAB3299
Anti-Fas (human, neutralizing), clone ZB4	05-338
Anti-Phosphatidylserine, clone 1H6, Alexa Fluor 488 conjugate	16-256
Anti-Phosphatidylserine, clone 1H6	05-719
Anti-Bcl2, clone 100	05-729
Anti-Cystatin C	06-458
Anti-Caspase 3, large subunit & proform	AB1899
Anti-Bim, internal epitope, pan-Bim isoforms	AB17003
Anti-AIF, internal domain	AB16501
Anti-Cathepsin D	06-467
Anti-Bim, clone 14A8	MAB17001-50UG
Anti-Caspase 8	AB1879
Anti-Caspase 3	06-735
Anti-Caspase 9, clone 96-2-22	05-572
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