New Apoptosis-detection Tools Permit Analysis of Cell Death-associated Events

Analyze apoptosis-induced DNA fragmentation, membrane phospholipid changes, and activation of ICE-like proteases with BM's new detection tools

Altered cellular processes can serve as indicators of apoptosis

Apoptosis, or programmed cell death, is associated with changes in several cellular processes. For example, it alters plasma membrane asymmetry, cleaves cellular DNA into histone-associated DNA fragments, and activates ICE-like proteases (Figure 1).

A growing product range of kits and reagents from Boehringer Mannheim allows researchers to study the effects of apoptosis on each of these cellular pathways. Now researchers can analyze multiple apoptosis parameters in order to confirm the results of apoptosis experiments.

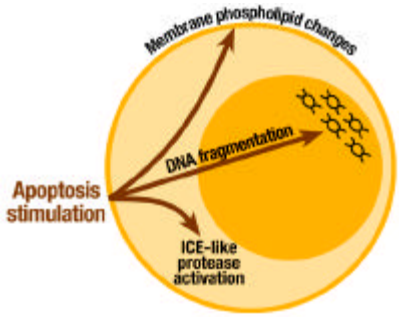


Figure 1 **Cellular processes that are altered in apoptosis and can be measured by Boehringer Mannheim products.**

Measure DNA fragmentation in early apoptotic cells

During early apoptosis, a Ca^{2+}-dependent endonuclease cleaves cellular DNA into histone-associated DNA fragments (mono- and oligo-nucleosomes) prior to the appearance of morphological changes. Boehringer Mannheim offers several kits based on three different methods for measuring DNA fragmentation as an indicator of apoptosis.

The convenient Cell Death Detection ELISA^{PLUS} measures nucleosomal particles in cytoplasmic fractions (lysates) or cell culture supernatants. Compared to the additional DNA ladder method, this kit requires 1000-fold fewer cells, eliminates the need for DNA isolation, and produces quantitative results in just 6–14 h.

The *In Situ* Cell Death Detection Kit, Fluorescein simplifies TUNEL (terminal deoxynucleotidyl transferase-mediate dUTP nick end labeling) assays. Incorporated fluorescein-dUTP is detected directly by flow cytometry or microscopy (Figure 2). The *In Situ* Cell Death Detection Kit, AP and *In Situ* Cell Death Detection Kit, POD allow simultaneous TUNEL-based apoptosis detection and morphological localization under a light microscope.

The Apoptotic DNA Ladder Kit purifies DNA from apoptotic cells for the detection of DNA ladders, the hallmark indicator of apoptosis. It eliminates organic extractions and DNA-precipitation steps. The kit's positive control simplifies analysis of the distinctive apoptotic DNA ladder.

Annexin V identifies apoptosis-associated plasma membrane lipid changes

Entry into apoptosis leads to the translocation of phosphatidyl serine from the inner leaflet to the extracellular side of the plasma membrane. Annexin V, a protein that binds to phosphatidyl serine with high affinity, can be used to detect apoptosis-induced membrane changes.

Annexin-V-FLUOS and Annexin-V-Biotin label apoptotic cells for quantification by flow cytometry. By staining cells with both the Annexin-V-FLUOS and propidium iodide provided in the Annexin-V-FLUOS Staining Set, it becomes possible to discriminate between apoptotic, necrotic, and normal cells in a cell suspension. Cell cultures can also be analyzed by using Annexin V in fluorescence or light microscopy.

Anti-PARP reveals apoptosis induced proteolytic fragments

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme involved in DNA repair. In many cell types, an early biochemical event in apoptosis is the proteolytic cleavage of PARP by an ICE-like (Interleukin-converting enzyme-like) protease acting at a highly conserved cleavage site. On a Western blot, Anti-PARP detects the resulting proteolytic PARP fragments in extracts from early apoptotic cells.

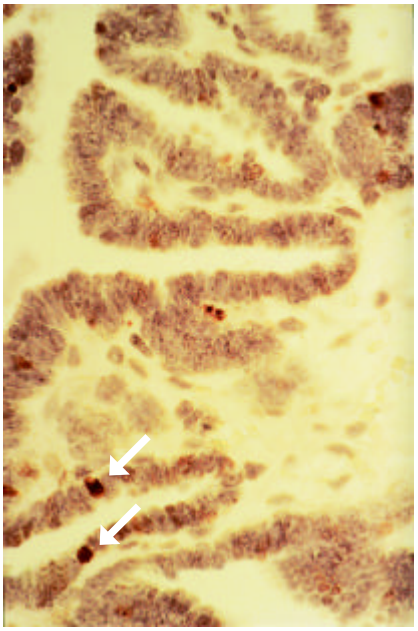


Figure 2 **Detection of apoptotic cells (indicated by arrows) in rabbit endometrium** by light microscopy with the *In Situ* Cell Death Detection Kit, POD.

Confirm apoptosis results by analyzing multiple parameters

For more information on Boehringer Mannheim's apoptosis-detection products, request *Cell Death: Tools for Apoptosis and Cytotoxicity Studies* from your local representative. Read about our latest apoptosis products (e.g., Anti-Fas antibodies) at http://biochem.boehringer-mannheim.com on the Internet.

Product	Cat. No.	Pack Size
Cell Death Detection ELISA^{PLUS}	1 774 425	96 tests
In Situ Cell Death Detection Kit, Fluorescein	1 684 795	50 tests
In Situ Cell Death Detection Kit, AP	1 684 809	50 tests

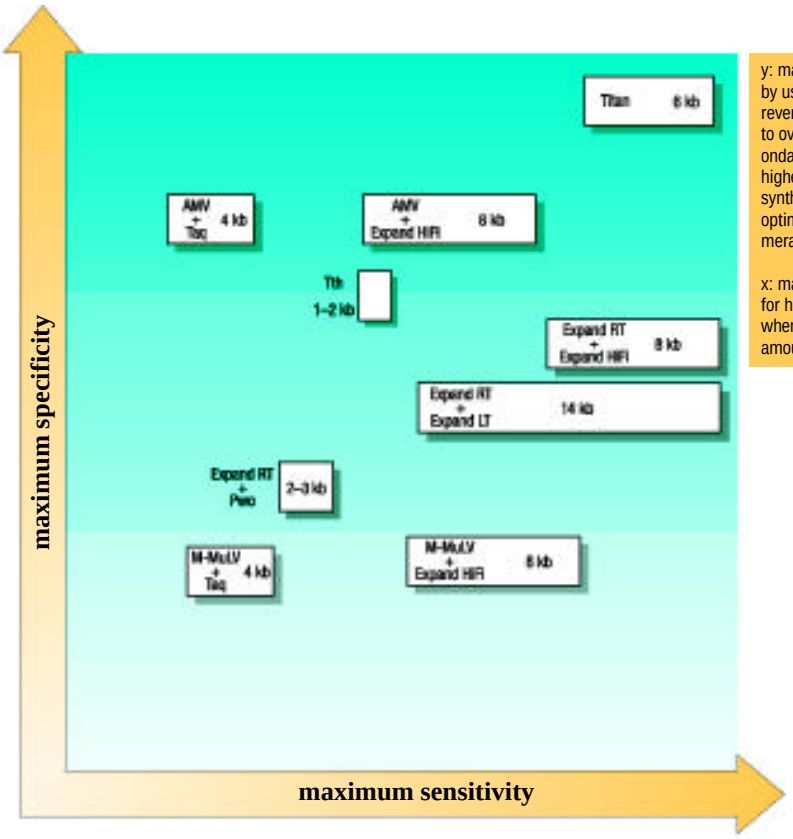
Product	Cat. No.	Pack Size
In Situ Cell Death Detection Kit, POD	1 684 817	50 tests
Apoptotic DNA-Ladder Kit	1 835 246	20 tests
Annexin-V-FLUOS	1 828 681	250 tests
Annexin-V-Biotin	1 828 690	250 tests
Annexin V FLUOS Staining Kit	1 858 777	for 50 tests
Anti-PARP	1 835 238	100 µl

For further information, see our folder "***Cell Death: Tools for Apoptosis and Cytotoxicity Studies.***"

Enzyme Combinations for RT-PCR

The combination of reverse transcriptase and the DNA polymerase system is very important for the results of One-step or Two-step RT-PCR, in terms of sensitivity, specificity, and fragments' size. Boehringer Mannheim is offering 9 recommended combinations and 3 kits to meet our customers' needs. The following schedule will help you to choose the right enzyme combination for your specific application in RT-PCR.

The First Strand cDNA Synthesis Kit, Titan™ One Tube RT-PCR Kit, and 5'/3' RACE Kit, which are not included in the schedule, are described on the bottom of this page.



y: maximum specificity by use of the optimum reverse transcriptase to overcome RNA secondary structures, and highest precision in synthesis/fidelity by optimum DNA polymerase

x: maximum sensitivity for highest yields, also when using very small amounts of probe.

M-MuLV + *Taq* DNA Polymerase	For maximum 4 kb fragments, sufficient specificity and sensitivity for routine applications
M-MuLV + Expand™ High Fidelity	Enhanced specificity and sensitivity for up to 8 kb fragments
Expand RT + *Pwo* DNA Polymerase	For 2-3 kb fragments, specificity and sensitivity in the mid range are guaranteed
Expand RT + Expand High Fidelity	Maximum sensitivity and high specificity for fragments up to 8 kb
Expand RT + Expand Long Template	High specificity and sensitivity up to 14 kb fragment size
***Tth* DNA Polymerase**	Easy One-step RT-PCR for routine application (1-2 kb) and carryover prevention
AMV + *Taq* DNA Polymerase	Guarantees high specificity reverse transcription for 4 kb fragments
AMV + Expand High Fidelity	Highest specificity and sensitivity for 8 kb fragments
Titan™ One Tube RT-PCR System	Enzyme mix and conditions optimized for fragments up to 6 kb
Titan One Tube RT-PCR Kit	Titan One Tube RT-PCR System + control RNA, for qualitative analysis and quantification of RT-PCR
First Strand cDNA Synthesis Kit	Complete kit with AMV, primers, dNTPs, and buffers; enables easy synthesis of a cDNA first strand
5'/3' RACE Kit	DNA amplification between an internal sequence and the 3' or 5' end, starting from mRNA