

# DeadEnd™ Colorimetric Apoptosis Detection System: Applications in Pathology



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*The genetic, molecular and morphological aspects of apoptosis currently are under intensive study because of the important role apoptosis has in development and homeostasis, as well as in several disease states. Promega's DeadEnd™ Colorimetric Apoptosis Detection System labels fragmented DNA, one of the biochemical hallmarks of apoptosis. This colorimetric system is ideal for labeling apoptotic nuclei in tissue sections and cultured cells, while allowing for concurrent morphological assessment. In this report, the DeadEnd™ Apoptosis System is demonstrated in situ using cellular and animal models of apoptosis and in multiple pathology tissue sections.*

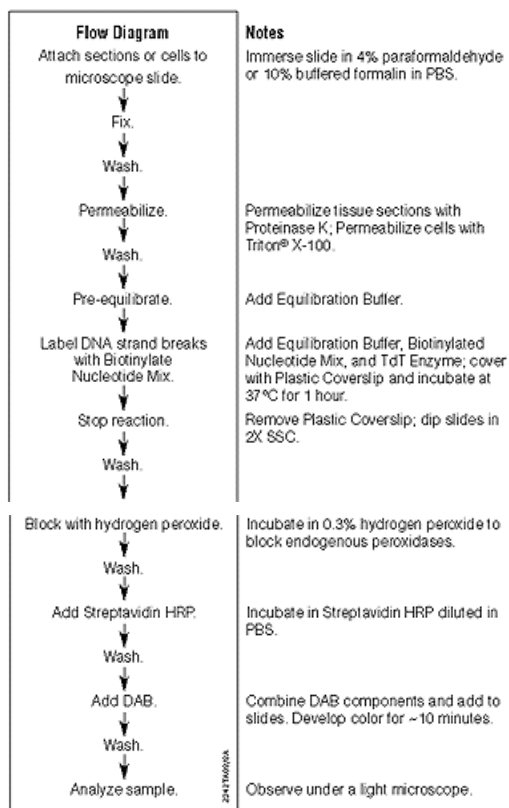
## INTRODUCTION

Apoptosis, a mechanism of cell suicide, is an intrinsic biological event that plays an essential role in development, homeostasis and in many disease processes. Culling extra cells in a precise and systematic way is an important aspect of normal development. Hence, the other term for the process is programmed cell death. However, in several diseases the cell death program goes awry. Degenerative diseases may result in (or be the result of) excessive apoptosis, and some cancers appear to inhibit cell death cascades resulting in excessive, uncontrolled proliferation.

Apoptosis was originally defined in terms of morphological changes, such as reduction in cell volume, condensation of the nucleus and membrane blebbing. Cells undergoing apoptosis fragment into membrane-bound apoptotic bodies that are readily phagocytosed and digested by macrophages or other neighboring cells without generating an inflammatory response. This is in contrast to necrosis, which results from gross insult to the cell, characterized by cell swelling, release of lysosomal enzymes, cellular disintegration and inflammation.

Apoptosis occurs in multiple cell types and can be triggered by multiple extracellular stimuli. With the discovery of several cell death genes conserved across numerous species, the cell death pathways are currently under intensive study. Morphological changes are observed in the nucleus of apoptotic cells, and DNA fragments are generated through the action of endogenous endonucleases (1,2) including the caspase-dependent DNase, CAD (3). This DNA fragmentation is one hallmark of apoptosis. Typically, the DNA of apoptotic cells is cleaved into a population of fragments, composed of multimers of 180-200bp in length. These multimeric fragments are readily observed on agarose gels as a "ladder."

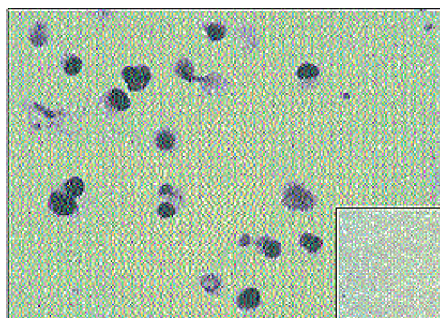
The DeadEnd™ Colorimetric Apoptosis Detection System end-labels the fragmented DNA of apoptotic cells. The system is based on a modified TUNEL, or TdT-mediated dUTP nick end-labeling, assay. [Figure 1](#) shows the steps of the assay. The DeadEnd™ System labels fragmented DNA *in situ* in both cultured cells and tissue sections (4). Using this procedure, apoptotic nuclei are stained dark brown.



**Figure 1. Protocol overview for use of the DeadEnd™ Colorimetric Apoptosis Detection System (4).**

## CELLULAR MODEL OF APOPTOSIS

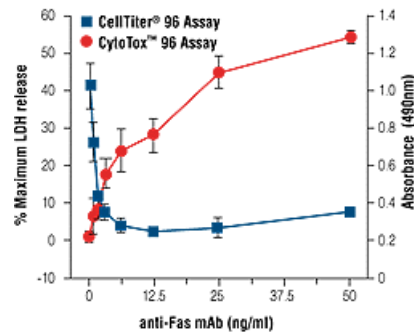
One well-established model of apoptosis is Fas-mediated cell death. This can be triggered by Fas-ligand (FasL) binding to the Fas receptor or by antibody cross-linking of the receptor (5,6). Fas-induced cell death is detectable within a few hours following ligand binding and does not require protein synthesis (5,7). It is believed that Fas ligation leads to activation of a protease cascade that ultimately results in transduction of a death signal (8). We have used antibody cross-linking of Fas in Jurkat cells, a human T cell lymphoma line, as a means to demonstrate the utility of Promega's new DeadEnd™ Colorimetric Apoptosis Detection System. As evident in [Figure 2](#), numerous cells are labeled with the DeadEnd™ Apoptosis Detection System after anti-Fas treatment, whereas few cells are labeled in the untreated controls.



**Figure 2. Apoptosis induced in Jurkat cells with an anti-Fas mAb (50ng/ml; Clone CH-11, Oncor).** The DeadEnd™ Colorimetric Apoptosis Detection System labels cell nuclei in the Fas mAb-treated cells (16 hours). Untreated cells are not stained (inset).

Also, Promega's CellTiter 96® AQ<sub>ueous</sub> One Solution Cell Proliferation<sup>(a)</sup> and CytoTox 96® Non-Radioactive Cytotoxicity Assays were used in tandem as an additional measure of cell viability 16 hours after the induction of apoptosis ([Figure 3](#)). The CellTiter 96® AQ<sub>ueous</sub> Assay measures the bioreduction of the tetrazolium salt, MTS<sup>(a)</sup>, as a means of quantifying cell viability (9). The CytoTox 96® Assay measures the release of lactate dehydrogenase (LDH) following disruption of cell membranes (10). The CellTiter 96® AQ<sub>ueous</sub> Assay revealed a decrease in the total number of viable cells with increasing amounts of Fas Ab added to Jurkat cells to induce apoptosis, while concomitantly, the CytoTox 96® Assay demonstrated an increase in the number of dead cells as shown in [Figure 3](#). The results of

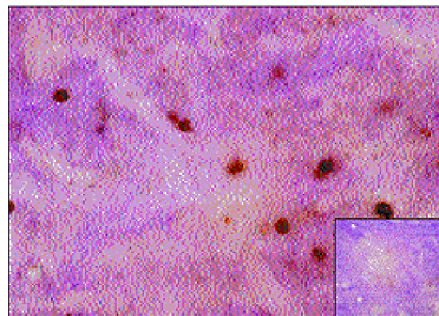
these two assays are consistent with the presence of apoptotic cells as determined using the DeadEnd™ System in this Fas-mediated Jurkat cell model of apoptosis (Figures 2 and 3). Similar results comparing the effectiveness of the CellTiter 96® AQ<sub>ueous</sub> Assay and the CytoTox 96® Assay as well as the CaspACE™ Assay System, Fluorometric, have been demonstrated for HL-60 cells induced to undergo apoptosis using anisomycin treatment (11).



**Figure 3. Apoptosis induced in Jurkat cells with varying concentrations of the anti-Fas mAb.** To induce apoptosis, 10<sup>6</sup> Jurkat cells/ml were incubated with a titration of 0-50ng/ml of anti-Fas mAb. After 16 hours at 37°C in 5% CO<sub>2</sub>, cell viability was measured with the CellTiter 96® AQ<sub>ueous</sub> One Solution Cell Proliferation Assay (absorbance at 490nm) and the CytoTox 96® Non-Radioactive Cytotoxicity Assay (% maximum LDH release) following the protocols described in the Technical Bulletins provided with each assay (9,10).

## ANIMAL MODEL OF APOPTOSIS

Animal models of apoptosis are useful for dissecting the pathways of programmed cell death *in vivo*. Axotomy-induced neuronal death in rat brain is an established model for studying apoptosis. A unilateral lesion of the visual cortex in the rat brain results in extensive neuronal cell death in the lateral geniculate nucleus (LGN) ipsilateral to the lesion (12,13). Neurons in the LGN are axotomized by the lesion, which results in their atrophy and death at a precise time following induction of the lesion. The time course of this axotomy-induced cell death demonstrates that, at 3-days post-lesion, only 5% of the neurons in the ipsilateral LGN have perished; however, between days 3 and 7 after the lesion, extensive neuronal death occurs. Approximately two-thirds of the neurons in the dorsal ipsilateral LGN undergo cell death during this time (12). In Figure 4, the DeadEnd™ Colorimetric Apoptosis Detection System discretely labels numerous nuclei in the LGN ipsilateral to the lesion, but not in the LGN contralateral to the lesion. These results clearly demonstrate the utility of this system for labeling apoptotic nuclei in the axotomy-induced neuronal death model.



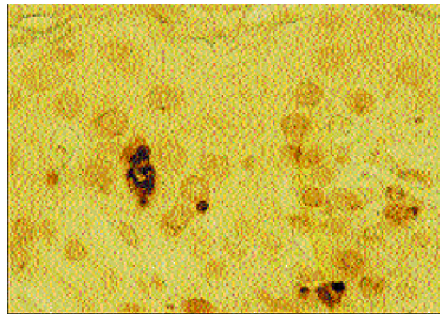
**Figure 4. The DeadEnd™ Colorimetric Apoptosis Detection System labels nuclei in the dorsal lateral geniculate nucleus (LGN) in a rat brain model of axotomy-induced apoptosis (11,12).** Vibratome sections (50µm) of paraformaldehyde/glutaraldehyde-fixed rat brains show several darkly stained cells in the LGN on the axotomized side of the brain, whereas there is no staining observed in the LGN on the contralateral side of the brain on the same section (inset). The "washboard" effect in the images is an artifact of the vibratome sectioning process.

## APOPTOSIS IN HUMAN DISEASE

Understanding the process of cell death associated with numerous disease states is essential for resolving the pathogenesis and identifying possible therapeutic approaches. In many diseases it is not obvious whether cell death occurs by apoptosis or by some other cytolytic path way. We have used the DeadEnd™ Apoptosis System to assess DNA fragmentation in several diseased tissues (below). This type of analysis can provide *in situ* information about the mechanism of cell death. Verification of apoptosis generally requires multiple lines of evidence, such as morphological criteria and a demonstration of nucleosomal degradation by DNA laddering methods. An analysis of DNA fragmentation with the DeadEnd™ Colorimetric Apoptosis Detection System can provide the first step toward understanding the mechanism of cell death in disease states.

## GRAFT VERSUS HOST DISEASE

Graft-versus-Host disease (GVHD) can be a serious complication of bone marrow transplants. In acute GVHD, the skin is a primary target organ for attack by infiltrating lymphocytes derived from donor bone marrow. Recently, excessive programmed cell death has been suggested to be involved in the destruction of keratinocytes in this immunologically mediated disease. The final pathway of target cell injury by effector T cells is now thought to be an apoptotic pathway in GVHD (14,15). In [Figure 5](#), the DeadEnd™ Colorimetric Apoptosis Detection System labeled cells in the basal cell layer in sections from a GVHD biopsy specimen that was formalin-fixed and paraffin-embedded. This is consistent with the idea that epidermal injury by effector lymphocytes results in apoptosis of keratinocytes in cutaneous GVHD.

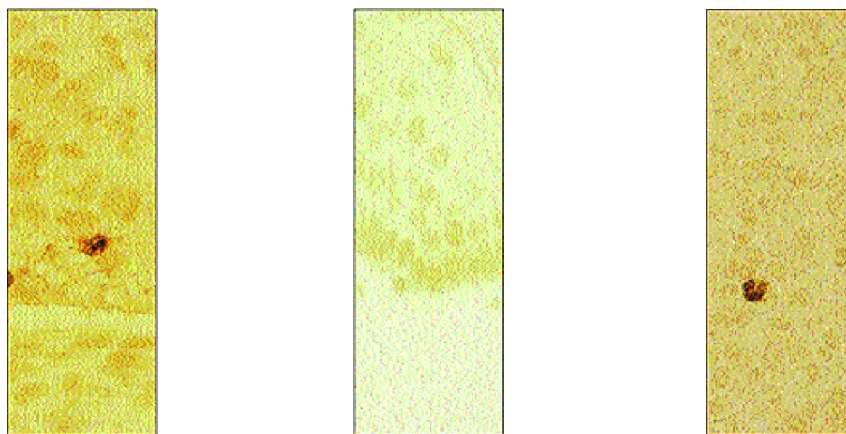


**Figure 5. The DeadEnd™ Colorimetric Apoptosis Detection System labels some cell nuclei of the basal cell layer of skin from a patient with Graft-versus-Host disease.** The sections are 5-10µm paraffin-embedded tissue samples. Sections were processed following the protocols described in the Technical Bulletin provided with the system (4).

## BASAL CELL CARCINOMA

Basal Cell Carcinoma (BCC) is a disease of the skin that can be triggered by solar UV-irradiation. Although BCC is a slow-growing, rarely metastatic tumor, the tumorous cells are able to invade and destroy the surrounding tissue. In this disease, inhibition of normal programmed cell death appears to occur. BCC is thought to be an actively proliferating lesion with a high apoptotic activity. The severity of the lesion depends on the rate of proliferation versus the rate of cell death. BCC cells express Bcl-2, which suppresses apoptosis (16,17). Differences in levels of Bcl-2 expression may affect the rate of cell death in basal cell carcinomas. In regressing BCC, the rate of cell death increases. It is thought that regression occurs by apoptosis (18).

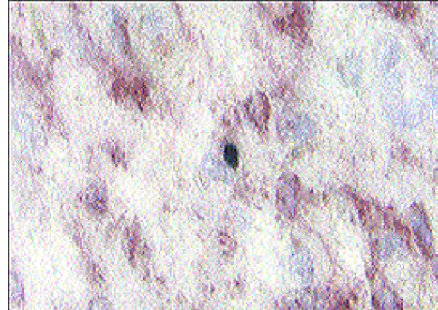
In [Figure 6](#), the DeadEnd™ Colorimetric Apoptosis Detection System was used to label numerous basal cell nuclei in sections of a regressing BCC. Staining of lymphocytic cells in regions surrounding the BCC lesions is also evident. Infiltrating peritumoral T lymphocytes are also thought to undergo apoptosis in BCC (19). Competition between cytotoxic T lymphocytes and the neoplastic basal cells may dictate the outcome of the disease.



**Figure 6. Detection of apoptosis in a regressing basal cell carcinoma.** In sections from a patient with a spontaneously regressing basal cell carcinoma, the DeadEnd™ Colorimetric Apoptosis Detection System labeled several nuclei in the basal cell layer in regions of the lesion (Panel A). Labeled nuclei were not observed in basal cells outside the lesions (Panel B). Several lymphocytic cells were also strongly labeled in regions adjacent to the lesions (Panel C). The basal cell layer tends to give higher background than the other tissues examined.

## MULTIPLE SCLEROSIS

Currently there is intensive study of the mechanisms underlying the elimination of oligodendrocytes and myelin from the demyelinated plaque of multiple sclerosis (MS). In this disease, apoptosis in demyelinated regions appears to play a significant role. Infiltrating cells and microglia undergo apoptosis in MS, but the cell death pathway for oligodendrocytes has not been definitively established (20-24). To identify the cell types undergoing DNA fragmentation in MS, and other diseases, colorimetric TUNEL techniques can be readily combined with antibody staining. Promega's DeadEnd™ Apoptosis Detection System was used to assess cell death in a demyelinated area of a hypercellular chronic stage MS lesion (Figure 7). The DeadEnd™ System was used to label apoptotic nuclei (black), and double-labeling with antibodies to myelin oligodendrocyte glycoprotein (MOG) was used to identify the oligodendrocytes (red). The apoptotic cell in Figure 7 is MOG-negative and thus is not an oligodendrocyte.



**Figure 7. Double-labeling reveals an apoptotic nucleus in a partially demyelinated area of a hypercellular chronic-stage multiple sclerosis lesion.** Nuclear fragmentation was visualized using the DeadEnd™ Colorimetric Apoptosis Detection System (with nickel enhancement, black), while the myelin segments and oligodendrocyte cell bodies were visualized with antibodies to myelin oligodendrocyte glycoprotein (MOG) (red). The apoptotic cell is MOG-negative and thus is not an oligodendrocyte. Cryosection was counterstained with haematoxylin (blue).

## SUMMARY

We have demonstrated the utility of the DeadEnd™ Colorimetric Apoptosis Detection System in cellular and animal models of apoptosis. This colorimetric system monitors DNA fragmentation while allowing morphological analysis of the cells or tissues. In addition, the DeadEnd™ Apoptosis System has proved to be extremely useful for assessing cell death in diseased tissue. This system can be combined with antibody staining for double-labeling with markers for particular cell types or other molecules in the pathways of apoptosis.

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## Ordering Information

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
DeadEnd™ Colorimetric Apoptosis Detection System	40 reactions	G7130
CellTiter 96® AQueous One Solution Cell Proliferation Assay	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581
CytoTox 96® Non-Radioactive Cytotoxicity Assay	1,000 assays	G1780

<sup>(a)</sup>The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida, which is licensed exclusively to Promega Corporation.

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