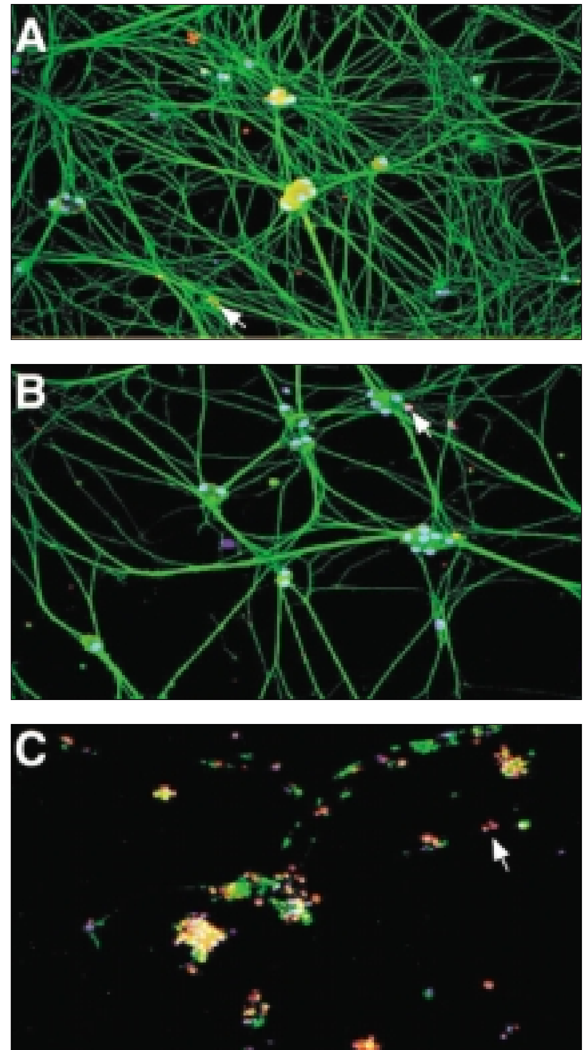
Apoptosis FAQs

How can I change the colors of a fluorescent TUNEL assay?

During apoptosis, chromosomal DNA is fragmented into 180–200bp fragments and is a late event of apoptosis (1). The apoptotic DNA may be analyzed by isolation and gel electrophoresis (2) or by end-labeling the fragments with the TUNEL assay (3). Promega has two systems that can be used to end-label the fragments—the Apoptosis Detection System, Fluorescein (Cat.# G3250), which uses the traditional fluorescein-dUTP[(a)] and the DeadEnd™ Colorimetric Apoptosis Detection System (Cat.# G7130, G7360), which uses a biotinylated nucleotide for end-labeling. The Apoptosis Detection System, Fluorescein, produces green fluorescence, and the DeadEnd™ System is most commonly used in conjunction with a streptavidin-HRP conjugate and DAB color development.

In some cases, such as with double- or triple-labeling, other fluorophores may be desired than just fluorescent green and the colorimetric brown. In the case of the traditional TUNEL assay, one can simply exchange the usual fluorescein-dUTP for some other labeled dUTP. For instance, Cy™3-dUTP can be substituted for fluorescein-dUTP in a standard TUNEL reaction (4). Thus, many different labels can be incorporated to arrive at any color desired.

An alternative is to end-label the fragmented DNA with a biotinylated nucleotide and react the cells with a streptavidin conjugate of the desired color. The latter strategy was used by Kohn *et al.* (2) to identify apoptotic cells in a sympathetic neuron culture. The cells were processed with the DeadEnd™ Colorimetric Apoptosis Detection System (Cat.# G7130), but at the point of addition of the streptavidin-HRP conjugate for color development, the researchers substituted a streptavidin-Cy™3 conjugate instead (Figure 1). The cells were treated with a FITC-labeled secondary antibody to identify α-tubulin staining (*green*), a Hoechst nuclear stain (*blue*) and the modified TUNEL assay with the streptavidin-Cy™3 detection (*red/pink*). This method allows tremendous flexibility in that the assay can be colorimetric (using streptavidin-HRP) or fluorescent (using any color tag and the appropriately labeled streptavidin).



▲ Figure 1.
Digitized micrographs are of postnatal day 1 sympathetic neuron cultures triple-labeled to visualize neurite outgrowth (α-tubulin, *green*), apoptotic cells (DeadEnd™ System, *red/pink*) and total cells (Hoechst nuclear stain, *blue*). Cultures were grown in 50ng/ml NGF for two days and then switched to either (A) 10ng/ml NGF, (B) 10ng/ml NGF plus 100ng/ml BDNF or (C) withdrawn from NGF. Cells were stained after two days of treatment. Magnification: 160X.

Images reprinted by kind permission of Drs. Judi Kohn and Freda Miller, Montréal Neurological Institute, and the Society for Neuroscience.

References

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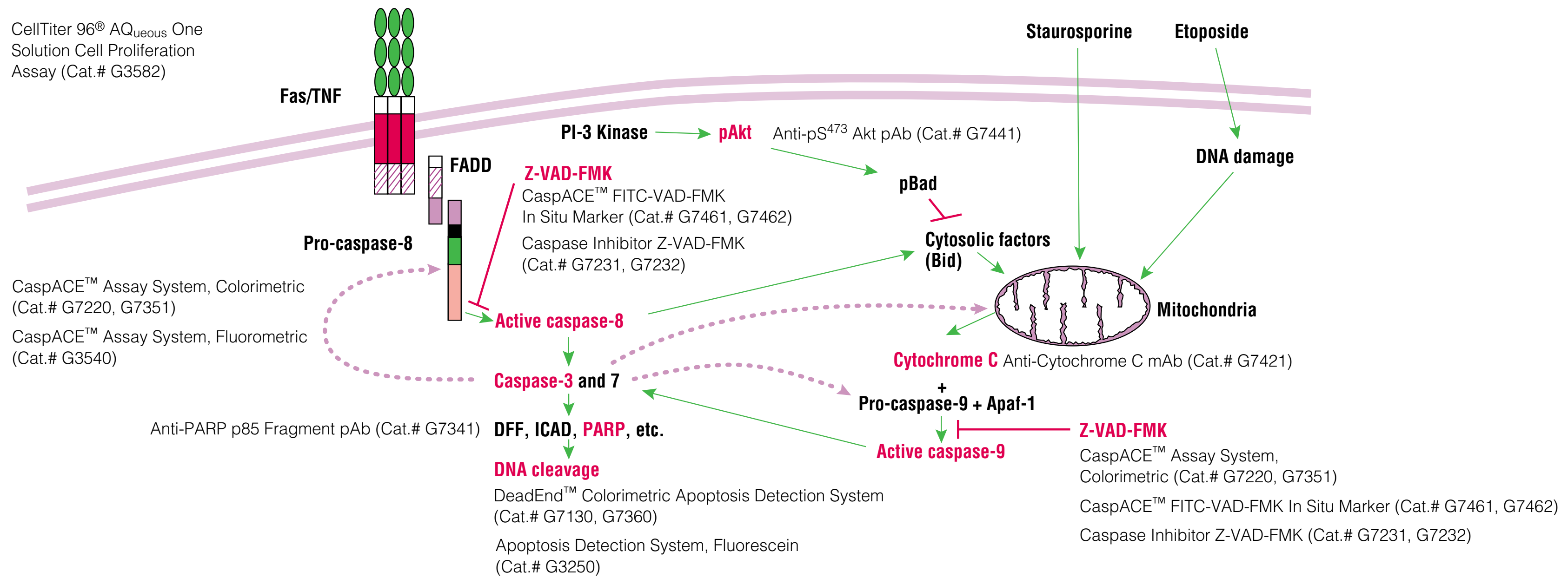
2. Arends, M.J. *et al.* (1990) *Am. J. Pathol.* **136**, 593.
3. Gavrieli, Y. *et al.* (1992) *J. Cell Biol.* **119**, 493.
4. Tornusciolo, D.R.Z. *et al.* (1995) *BioTechniques* **19**, 800.



▲ **The Jurkat cell apoptosis model system**

This schematic diagram illustrates the key role that Akt plays in anti-apoptotic events. Relevant Promega products are noted. The figure is a modification of a figure that originally appeared in Sun, X.M. *et al.* (1999) *J. Biol. Chem.* **274**, 5053.

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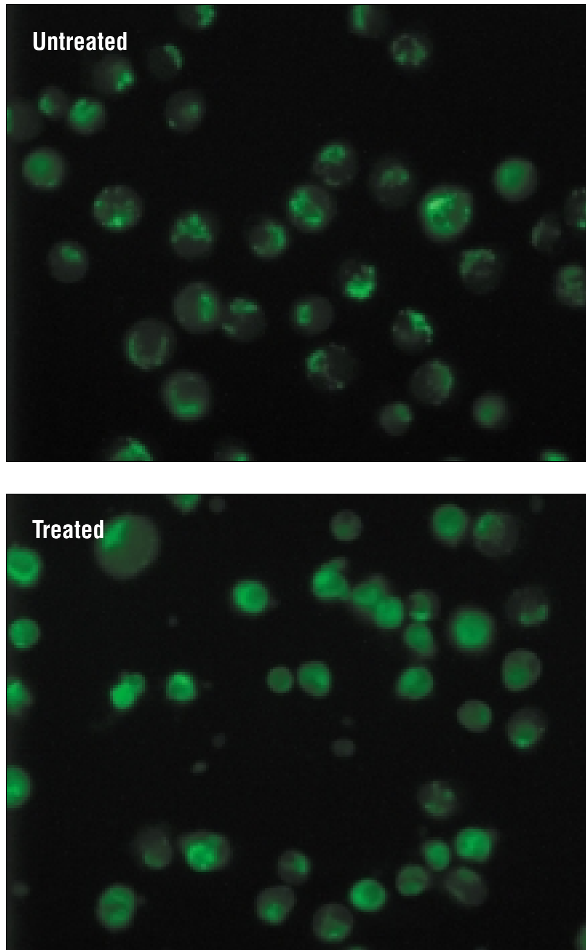
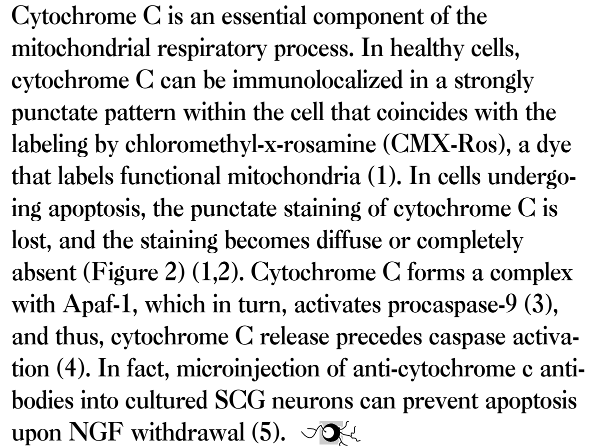


▲ Figure 2.
Jurkat cells untreated (top) or treated (bottom) with an anti-Fas mAb (PanVera) for 5 hours. The cells were fixed and stained with 1.0µg/ml Anti-Cytochrome C mAb. Note that, after anti-Fas treatment, the labeling for cytochrome C is more diffuse than for untreated cells.

What differentiates an apoptotic cell from a normal cell with regard to immunostaining with Anti-Cytochrome C mAb?



Cytochrome C is an essential component of the mitochondrial respiratory process. In healthy cells, cytochrome C can be immunolocalized in a strongly punctate pattern within the cell that coincides with the labeling by chloromethyl-x-rosamine (CMX-Ros), a dye that labels functional mitochondria (1). In cells undergoing apoptosis, the punctate staining of cytochrome C is lost, and the staining becomes diffuse or completely absent (Figure 2) (1,2). Cytochrome C forms a complex with Apaf-1, which in turn, activates procaspase-9 (3), and thus, cytochrome C release precedes caspase activation (4). In fact, microinjection of anti-cytochrome c antibodies into cultured SCG neurons can prevent apoptosis upon NGF withdrawal (5).

References

1. Krohn, A.J. *et al.* (1999) *J. Neurosci.* **19**, 7394.
2. Srinivasan, A. *et al.* (1998) *J. Biol. Chem.* **273**, 4523.
3. Zou, H. *et al.* (1999) *J. Biol. Chem.* **274**, 11549.
4. Stefanis, L. *et al.* (1999) *J. Neurosci.* **19**, 6235.
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Ordering Information

Product	Size	Cat.#
Apoptosis Detection System, Fluorescein	60 reactions	G3250
DeadEnd™ Colorimetric Apoptosis Detection System	40 reactions	G7130
	20 reactions	G7360
Anti-Cytochrome C pAb	100µg	G7421

Related Products	Size	Cat.#
CaspACE™ Assay System, Colorimetric	100 assays	G7220
	50 assays	G7351
CaspACE™ Assay System, Fluorometric	160 assays	G3540
CaspACE™ FITC-VAD-FMK In Situ Marker	50µl	G7461
	125µl	G7462
Caspase Inhibitor Z-VAD-FMK	50µl	G7231
	125µl	G7232
Anti-pS473 Akt pAb	40µl	G7441
Anti-PARP p85 Fragment pAb(b)	50µl	G7341

(a)Manufactured for Promega Corporation by NEN® Life Science Products under U.S. Pat. Nos. 5,047,519 and 5,151,507.
(b)Patent Pending.