BD Clontech ApoAlert™ pCaspase3-Sensor Vector

Monitor caspase-3 activity in a single cell

- **Easily detect caspase-3 activity by fluorescence microscopy**
- **Real-time monitoring of living cells using BD Living Colors™ Enhanced Yellow Fluorescent Protein**
- **Quickly determine induction of apoptosis in an individual cell**

Now you can monitor caspase-3 activity in individual cells using our **BD Clontech ApoAlert™ pCaspase3-Sensor Vector**†. Following a simple transfection, this vector construct lets you visualize caspase-3 activity in real-time on a cell-to-cell basis. Through fluorescence microscopy you now have the tools to detect caspase-3 activation in individual cells.

Novel vector design

The basis of the novel vector is the unique Caspase3-Sensor protein (Figure 1). This protein contains a dominant, N-terminal nuclear export signal (NES), an optimized caspase-3 cleavage site, BD Living Colors™ Enhanced Yellow Fluorescent Protein (EYFP), and a C-terminal nuclear localization signal (NLS).

How the Caspase3-Sensor works

When caspase-3 is not active in the cell, the dominant NES sequence targets the EYFP to the cytosol. When caspase-3 is activated, it cleaves off the NES sequence, allowing the NLS to translocate EYFP to the nucleus (Figure 2). This translocation can be easily detected using fluorescence microscopy (Figure 3).

Study caspase-3 activity in individual cells

In contrast to standard assays, the Caspase3-Sensor lets you detect caspase activity in individual cells. This means you can differentiate among cells in a single population—the information you receive is richer and more complex. For example, a single population of apoptotic cells may be extremely heterogenous, consisting of cells in which caspase's activity is either high, low, or completely absent. Clues to why these differences exist can only be found by studying individual cells. Using our vector, you can

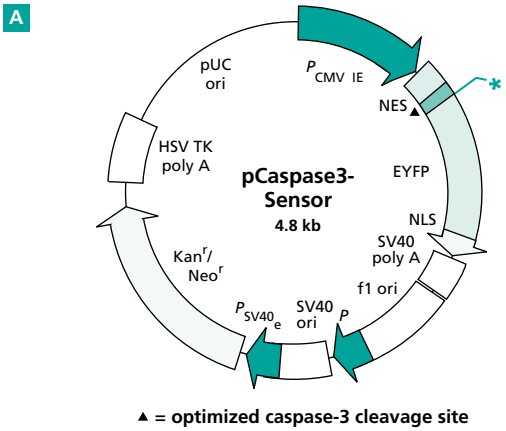

Figure 1. Caspase3-Sensor. Panel A. Vector Map. **Panel B.** Caspase3-Sensor protein. NES = nuclear export signal. DEVD = optimized caspase-3 cleavage sequence. EYFP = Enhanced Yellow Fluorescent Protein. NLS = nuclear localization signal. * = caspase cleavage site.

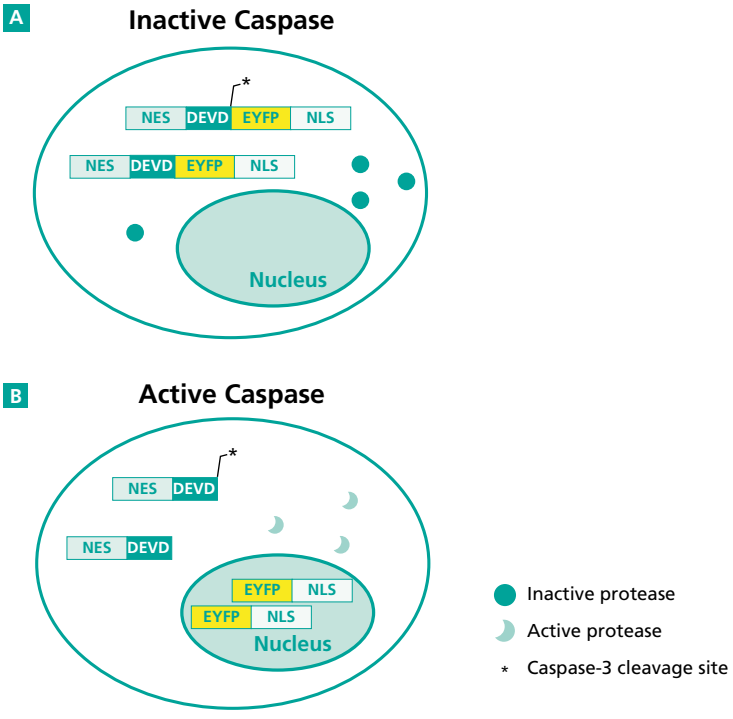

Figure 2. Schematic of the Caspase3-Sensor translocation in nonapoptotic cells and in apoptotic cells. When caspase-3 is inactive, the dominant nuclear export signal (NES) keeps the fluorescent protein in the cytosol (**Panel A**). When caspase-3 is active, the NES is cleaved from the protein and the fluorescent protein translocates to the nucleus (**Panel B**).

† Patent pending

pCaspase3-Sensor Vector...continued

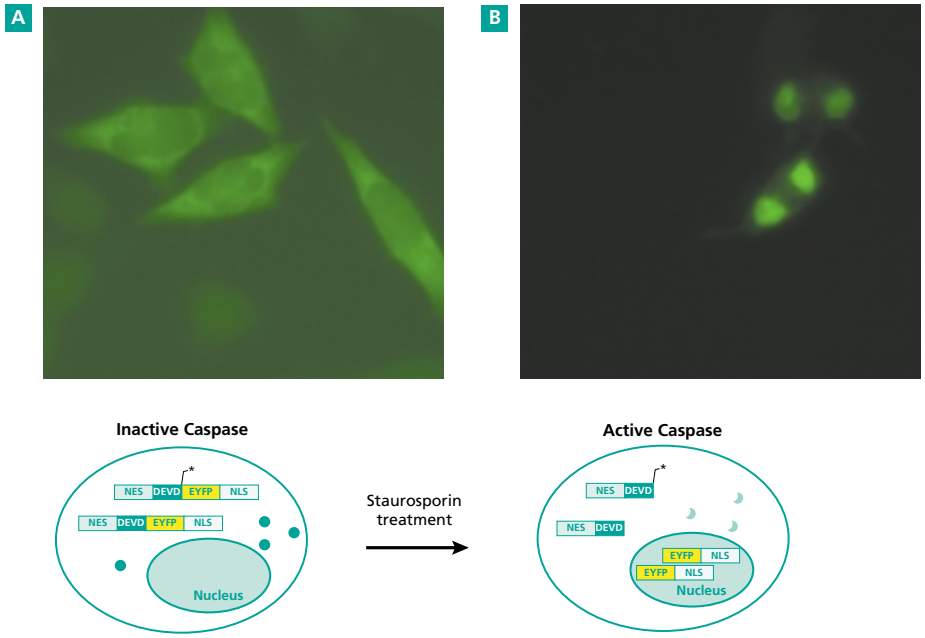

Figure 3. Single-cell detection of apoptosis using the BD Clontech ApoAlert™ pCaspase3-Sensor Vector. 3T3 cells were grown on coverslips, transiently transfected with pCaspase3-Sensor, and grown for 24 hrs. Apoptosis was induced using staurosporin (700 nM) and caspase-3 activity was detected 4 hrs post induction. Cells were fixed with 3% paraformaldehyde and photomicrographs were taken using a Zeiss microscope. **Panel A.** 3T3 cells transfected with pCaspase3-Sensor (untreated control). **Panel B.** 3T3 cells transfected with pCaspase3-Sensor treated with staurosporin.

determine if caspase-3 is active within a single cell. Simply transfect our pCaspase3-Sensor Vector into the mammalian cell of your choice, induce apoptosis, and monitor the response of individual cells using fluorescence microscopy. You can also cotransfect other expression plasmids with the pCaspase3-Sensor Vector to investigate the influence of your protein on caspase-3 activation.

In Figure 3, we show the induction of caspase-3 activity in 3T3 cells transfected with pCaspase3-Sensor Vector after treatment with staurosporin. When caspase-3 is not active, Caspase3-Sensor is distributed only in the cytosol (Panel A); however, in cells treated with staurosporin, activated caspase-3 cleaves off the NES and the remaining protein, containing EYFP, translocates to the nucleus (Panel B).

With the pCaspase3-Sensor Vector, BD Biosciences Clontech provides a new tool for studying apoptosis on a cellular level.

Product	Size	Cat. #
pCaspase-3 Sensor Vector	20 µg	8185-1

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