CellEvent™ Caspase-3/7 Green Detection Reagent

Catalog no. C10423

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
CellEvent™ Caspase-3/7 Green Detection Reagent	100 µL	2.0 mM solution in DMSO	• ≤–20°C • Protect from light	When stored as directed, the product is stable for 3 months from the date of receipt.
Approximate fluorescence excitation and emission maxima: 502/530 nm.				

Introduction

CellEvent™ Caspase-3/7 Green Detection Reagent is a novel fluorogenic substrate for activated caspases 3 and 7. The reagent consists of a four amino acid peptide (DEVD) conjugated to a nucleic acid binding dye. This cell-permeant substrate is intrinsically non-fluorescent, because the DEVD peptide inhibits the ability of the dye to bind to DNA. After activation of caspase-3 or caspase-7 in apoptotic cells, the DEVD peptide is cleaved, enabling the dye to bind to DNA and produce a bright, fluorogenic response with an absorption/emission maxima of ~502/530 nm (Figure 1).

There are several benefits of the CellEvent™ Caspase-3/7 Green Detection Reagent compared to other methods of detecting activated caspase 3/7. One important advantage of this assay is that no wash steps are required; this helps preserve the fragile apoptotic cells typically lost during wash steps. To use the CellEvent™ Caspase-3/7 Green Detection Reagent, add the substrate to your cells in complete growth medium or buffer, incubate for 30 minutes, and image (Figure 2). Apoptotic cells with activated caspase-3/7 show bright green nuclei, while cells without activated caspase 3/7 exhibit minimal fluorescence signal (Figure 3, page 2).

Figure 1 Fluorescence excitation and emission spectra of CellEvent™ Caspase-3/7 Green Detection Reagent after reaction with activated caspase-3 or 7, bound to DNA

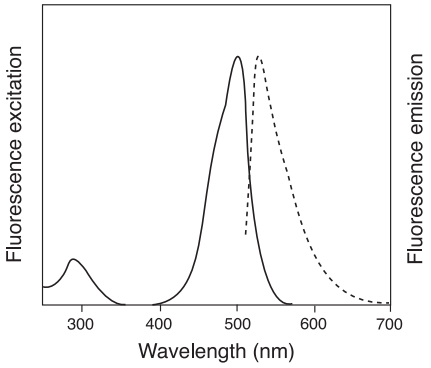
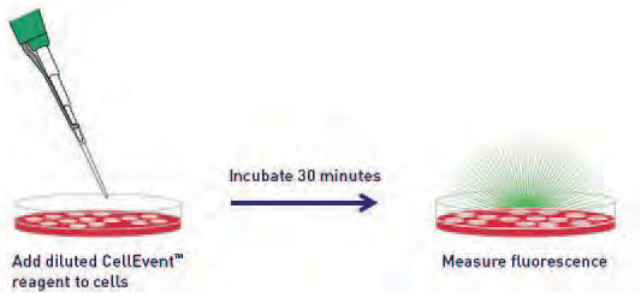


Figure 2 Workflow for the CellEvent™ Caspase-3/7 Green Detection Reagent



This robust assay is highly specific for caspase-3/7 activation (Figure 4) and can be used to monitor caspase-3 or -7 activation with live-cell fluorescence imaging. Because the cleaved reagent labels nuclei of caspase 3/7–positive cells, this stain can also provide information on nuclear morphology, including condensed nuclei typical of late-stage apoptosis. Additionally, the fluorescent signal from CellEvent™ Caspase-3/7 Detection Reagent survives formaldehyde fixation and detergent permeabilization. This provides flexibility in assay workflow and extends the multiplexibility of this probe for the detection other proteins of interest using immunocytochemistry.

In addition to traditional fluorescence microscopy, the CellEvent™ Caspase-3/7 Green Detection Reagent has been validated for high-content imaging and analysis. The drastic change in fluorescence between normal and apopotic cells within a population provides an excellent assay window, and the z-factor value indicates the reagent is robust enough for use in high-content imaging assays (Figure 5).

Figure 3 HeLa cells were loaded with 7.5 µM CellEvent™ Caspase-3/7 Green Detection Reagent then treated with 0.5 µM staurosporine (right) or vehicle control (left) for 4 hours. Staurosporine-induced apoptosis was detected with CellEvent™ Caspase-3/7 Green Detection Reagent. Apoptotic cells (right) fluoresce bright green, while non-apoptotic control cells do not show any signal (left).

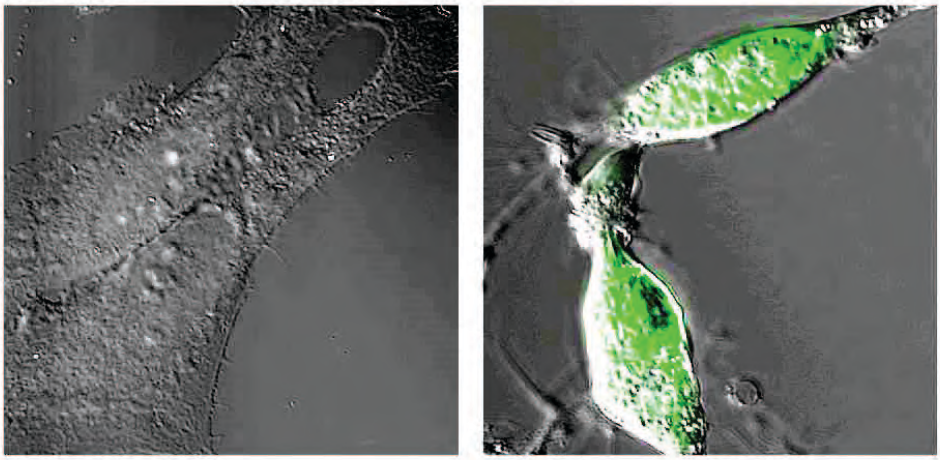


Figure 4 To determine the specificity of CellEvent™ Caspase-3/7 Green Detection Reagent, HeLa cells were treated with 0.5 µM staurosporine in the presence or absence of the Caspase 3/7 Inhibitor 1 (EMD Chemicals) at 0–30 µM for 4 hours. Cells were then labeled with 5 µM CellEvent™ Caspase-3/7 Green Detection Reagent, followed by Hoechst 33342 for 15 minutes in complete medium. Images were acquired and analyzed on a Thermo Fisher Cellomics ArrayScan® VTI. Quantitative analysis revealed a decrease in the percent of cells positive for active caspase 3/7 with increasing concentrations of inhibitor.

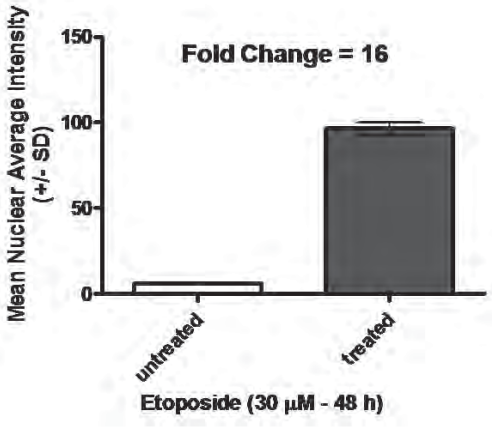
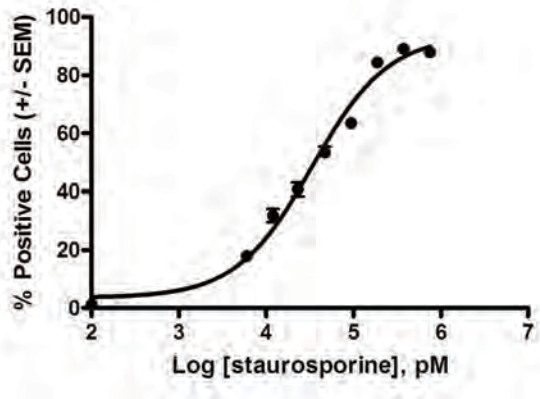


Figure 5 To generate a dose response curve, U-2 OS cells were plated onto a 96-well plate and treated with 0–0.75 µM staurosporine or vehicle control for 24 hours. Cells were then labeled with 7.5 µM CellEvent™ Caspase-3/7 Green Detection Reagent for 30 minutes at 37°C followed by Hoechst 33342 stain. Cells were analyzed on a Thermo Fisher Cellomics Arrayscan® VTI. The percent of cells positive for active caspase 3/7 was determined, and the EC50 was calculated.



Before Starting

Materials required but not provided

- Cells
- Complete medium or buffer suitable for the cell type used
- *Optional:* Fixative (i.e., 3.7% formaldehyde in PBS)

Caution

DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations. In case of contact with eyes, rinse immediately and thoroughly with water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling this reagent.

Experimental Protocols

The following protocol was developed with HeLa and U-2 OS cells with an optimized concentration of 5 µM, but it can be adapted for any cell type. Growth medium, cell density, cell type variations, and other factors may influence labeling. In initial experiments, we recommend testing a concentration range of CellEvent™ Caspase-3/7 Green Detection Reagent to determine the optimal conditions for your model.

Staining protocol

1. Add CellEvent™ Caspase-3/7 Green Detection Reagent at a final concentration of between 2–8 µM to the sample and/or appropriately induced cells and incubate for 30 minutes at 37°C. We recommend initial testing between 2–10 µM of CellEvent™ Caspase-3/7 Green Detection Reagent; however, optimal concentration may be more or less depending on model.

   Note: It is best to prepare an intermediate dilution of CellEvent™ Caspase-3/7 Detection Regent in complete medium, mix it well by pipetting, and then add the diluted solution to the cells so that the final concentration of the reagent on the cells is 5 µM. Incubation can also be performed successfully at room temperature (25°C).

2. *Optional:* Cells can be preserved with a formaldehyde-based fixative at this stage. Fixation with 3.7% formaldehyde for 15 minutes is recommended, but this can be altered based on the cell type.

3. *Optional:* Cells can be stained with a nuclear or other counterstain at this step.

4. *Optional:* To stabilize and prolong the signal, ProLong® Gold antifade reagent (Cat. no. P36934) can be used for ultimate overnight mounting. For quick mounting, SlowFade® Gold antifade reagent (Cat. no. S36937) can be used.

6. Image the cells using the appropriate instrument filter sets such as those used for FITC and the Alexa Fluor® 488 dye. The excitation/emission maxima for the CellEvent™ Caspase-3/7 Green Detection Reagent is 502/530 (Figure 1, page 1).

Product List Current prices may be obtained at www.invitrogen.com or from our Customer Service Department.

Catalog no.	Product name	Unit Size
C10423	CellEvent™ Caspase-3/7 Green Detection Reagent *2 mM solution in DMSO*	100 µL
C10422	CellROX™ Deep Red Reagent *for oxidative stress detection*	5 × 50 µL
	Related Products	
C10422	CellROX™ Deep Red Reagent *for oxidative stress detection*	5 × 50 µL
H3570	Hoechst 33342, trihydrochloride, trihydrate *10 mg/mL solution in water*	10 mL
T668	tetramethylrhodamine, methyl ester, perchlorate (TMRM)	25 mg
L10382	LC3B Antibody Kit for Autophagy *rabbit polyclonal LC3B* *includes autophagosome inducer*	1 kit

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