CellEvent™ Caspase-3/7 Green Detection Reagent

Catalog No. C10423, C10723

Pub. No. MAN0003556 **Rev.** B.0

Table 1. Contents and storage

Material	Amount		Concentration	Storage*
	C10423	C10723		
CellEvent™ Caspase-3/7 Green Detection Reagent	100 µL	25 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, page 2).

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 it does not require wash steps, which 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, page 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).

This robust assay is highly specific for caspase-3/7 activation (Figure 4, page 2) 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, page 2).

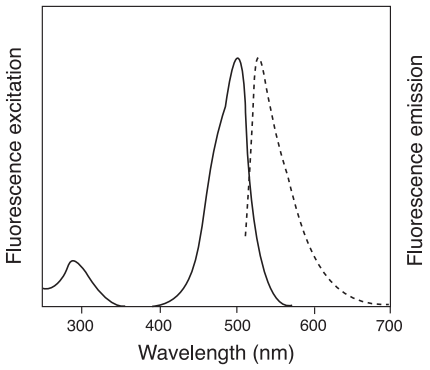


Figure 1. Fluorescence excitation and emission spectra of CellEvent™ Caspase-3/7 Green Detection Reagent after reaction with activated caspase-3 or 7, bound to DNA.

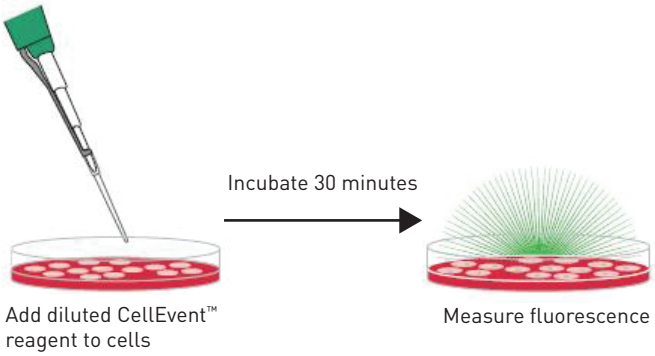


Figure 2. Endpoint assay workflow using the CellEvent™ Caspase-3/7 Green Detection Reagent.

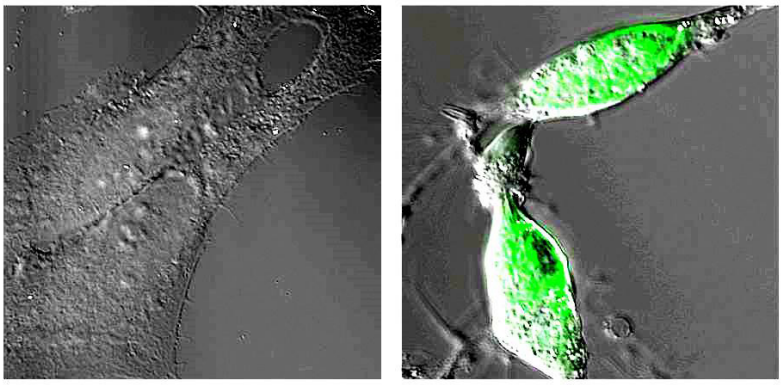


Figure 3. HeLa cells were loaded with 7.5 µM CellEvent™ Caspase-3/7 Green Detection Reagent then treated with 0.5 µM staurosporine (right) or vehicle control (left) for 4 hours. Staurosporine-induced apoptosis was detected with CellEvent™ Caspase-3/7 Green Detection Reagent. Apoptotic cells (right) fluoresce 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.

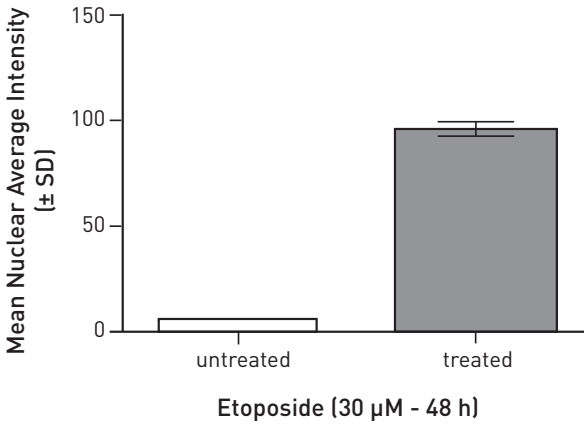
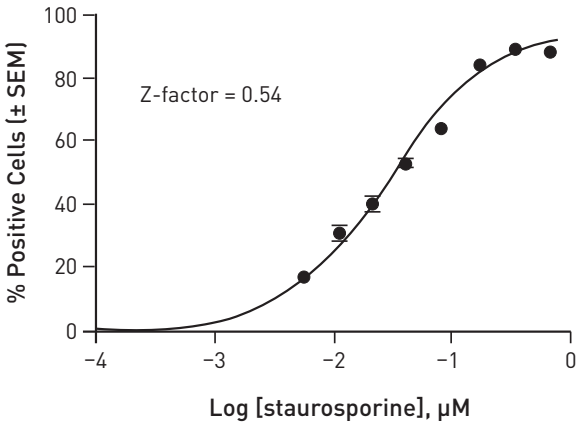


Figure 5. To generate a dose response curve, U-2 OS cells were plated onto a 96-well plate and treated with 0–0.75 µM staurosporine or vehicle control for 24 hours. Cells were then labeled with 7.5 µM CellEvent™ Caspase-3/7 Green Detection Reagent for 30 minutes at 37°C followed by Hoechst 33342 stain. 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 supplied

- Cells
- Fetal Bovine Serum, certified, heat inactivated (Cat. No. 10082147)
- DPBS, with calcium and magnesium (Cat. No. 14040133)
- *Optional*: Complete medium (as a diluent for CellEvent™ Caspase-3/7 reagent)
- *Optional:* Fixative (e.g. 3.7% formaldehyde in PBS)
- *Optional:* ProLong™ Diamond Antifade Mountant (Cat. No. P36970) or SlowFade™ Diamond Antifade Mountant (Cat. No. S36972)

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.

Methods

The following protocols were developed using HeLa and U-2 OS cells with an optimized CellEvent™ Caspase-3/7 Green Detection Reagent concentration of 5 µM, but they 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 range of concentrations for the CellEvent™ Caspase-3/7 Green Detection Reagent to determine the optimal conditions for your model.

Assay choice

The protocol for the endpoint apoptotic assay is provided below. However, you can also perform kinetic or dynamic measurement of the induction of apoptosis by treating the cells in complete medium with the CellEvent™ Caspase-3/7 Green Detection Reagent and incubating them for extended periods of time (page 4).

Endpoint assay

1. Treat cells with the appropriate apoptotic inducer for the desired time.
2. Dilute the CellEvent™ Caspase-3/7 Green Detection Reagent into PBS with 5% FBS (Cat. Nos. 14040133 and 10082147) or complete medium (see Note below) to a final concentration of 2–8 µM.

   Note: For best results, dilute the CellEvent™ Caspase-3/7 Reagent into PBS with 5% FBS. You can dilute the reagent into complete medium; however, this can result in high fluorescence background. We recommend that you perform optimization, if the preferred diluent is complete medium.

   We recommend initial testing with 2–10 µM of CellEvent™ Caspase-3/7 Green Detection Reagent. However, the optimal concentration may be more or less than this range depending on the model.

3. *Optional:* You can stain the cells with a cell permeable nuclear stain at this step.

4. Remove the media from the cells, then add the diluted reagent (prepared in Step 2) to the cells. For example, if you are performing staining in a 96-well plate, add 100 µL of the reagent solution to each well.

5. Incubate the cells at 37°C for at least 30 minutes.

   Note: It is best to determine the incubation time for the cell type of choice; in HeLa cells, 30 minutes is sufficient.

6. *Optional:* You can preserve the cells 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.

7. *Optional*: You can stain the cells with a nuclear stain or counterstain at this step, if they have not been previously counterstained.

8. *Optional*: To stabilize and prolong the signal, you can use ProLong™ Diamond antifade reagent (Cat. No. P36970) for ultimate overnight mounting. For quick mounting, you can use SlowFade™ Diamond antifade reagent (Cat. No. S36972).

9. 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 nm (Figure 1, page 1).

Kinetic assay

1. Dilute the CellEvent™ Caspase-3/7 Green Detection Reagent into complete medium to a final concentration of 2–10 µM.

   Note: We recommend initial testing with 2–10 µM of CellEvent™ Caspase-3/7 Green Detection Reagent. However, the optimal concentration may vary depending on the experimental conditions.

2. Prepare the apoptotic inducer.

3. Add the CellEvent™ Caspase-3/7 Green Detection Reagent prepared in complete medium (Step 1) directly to the cells in complete medium.

4. Add the apoptotic inducer to the cells treated with the CellEvent™ Caspase-3/7 Green Detection Reagent and return the cells to the incubator.

5. At the desired time points, remove the cells from the incubator and visualize the progression of apoptosis using FITC/Alexa Fluor™ 488 filter settings.

Product list

Cat. No.	Product name	Unit size
C10423	CellEvent™ Caspase-3/7 Green Detection Reagent *2 mM solution in DMSO*	100 µL
C10723	CellEvent™ Caspase-3/7 Green Detection Reagent *2 mM solution in DMSO*	25 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
P36970	ProLong™ Diamond Antifade Mountant	10 mL
S36972	SlowFade™ Diamond Antifade Mountant	10 mL
14040133	DPBS with calcium and magnesium	500 mL
10082147	Fetal Bovine Serum, certified, heat inactivated	500 mL

Documentation and support

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining support
For the latest services and support information for all locations, go to **thermofisher.com/support**.

At the website, you can:
- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
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- Submit a question directly to Technical Support (**thermofisher.com/support**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS
Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

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Limited Product Warranty
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Revision history: Pub. No. MAN0007522

Revision	Date	Description
B.0	10 January 2017	Add Kinetic Assay Protocol.
A.0	02 June 2016	Information on Cat. No. C10723 added, staining protocol updated.
1.0	07 February 2011	New document

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Corporate entity
Life Technologies | Carlsbad, CA 92008 USA | Toll free in USA 1.800.955.6288

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