CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit

Catalog Numbers C10427, C10740

Pub. No. MAN0007522 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

A distinctive feature of the early stages of apoptosis is the activation of caspase enzymes, which are cysteine-aspartic acid-specific proteases. These enzymes participate in a series of reactions triggered in response to pro-apoptotic signals and result in the cleavage of protein substrates and subsequent cell disassembly. The recognition sequence in the target substrate always includes an aspartic acid residue; cleavage takes place at the carbonyl end of that residue.

CellEvent™ Caspase-3/7 Green Detection Reagent is a fluorogenic substrate for detection of activated caspases 3 and 7 in apoptotic cells. This cell-permeant reagent consists of a 4 amino acid peptide (DEVD) conjugated to a nucleic acid binding dye. During apoptosis, caspase-3 and caspase-7 proteins are activated and are able to cleave the caspase 3/7 recognition sequence encoded in the DEVD peptide. Cleavage of the recognition sequence and binding of DNA by the reagent labels apoptotic cells with a bright, fluorogenic signal. When used together with the SYTOX™ AADvanced™ Dead Cell Stain, apoptotic cells can easily be discriminated from live and necrotic cells (see Figure 1).

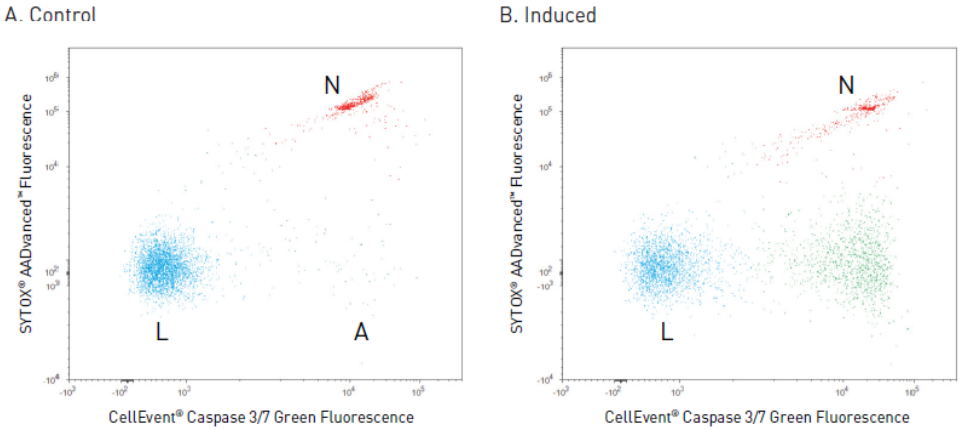

Figure 1 Flow cytometry analysis with the CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit. Jurkat cells (T-cell leukemia, human) were treated with (A) DMSO or (B) 10 µM camptothecin for 3 hours before labeling with the CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit. Stained samples were analyzed on the Attune™ Acoustic Focusing Cytometer equipped with a 488-nm laser, and fluorescence emission was collected using a 530/30 bandpass filter for CellEvent™ Caspase-3/7 Green Detection Reagent and a 690/50 bandpass filter for SYTOX™ AADvanced™ Dead Cell Stain, respectively. Note that the treated cells have a higher percentage of apoptotic cells (panel B) than the basal level of apoptosis seen in the control cells (panel A). A = apoptotic cells, V = viable cells, N = necrotic cells.

Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. Thermo Fisher Scientific offers a wide selection of products for apoptosis research; for more information, refer to http://thermofisher.com/apoptosis.

Spectral characteristics

The absorption and fluorescence emission spectra of the CellEvent™ Caspase-3/7 Green Detection Reagent following cleavage of the DEVD peptide sequence and SYTOX™ AADvanced™ Dead Cell Stain are given in Figure 2 (panels A and B, respectively). These spectra were obtained in 10 mM Tris, 1 mM EDTA, pH 8 in the presence of double-stranded DNA. The CellEvent™ Caspase-3/7 Green Detection Reagent exhibits greater than a 30-fold increase upon cleavage and binding DNA. Similarly, the SYTOX™ AADvanced™ Dead Cell Stain exhibits a fluorescence enhancement of greater than 500-fold. The absorption and fluorescence emission maxima of the CellEvent™ reagent/DNA and SYTOX™ AADvanced™ stain/DNA complexes are 511 nm/533 nm and 546 nm/647 nm, respectively.

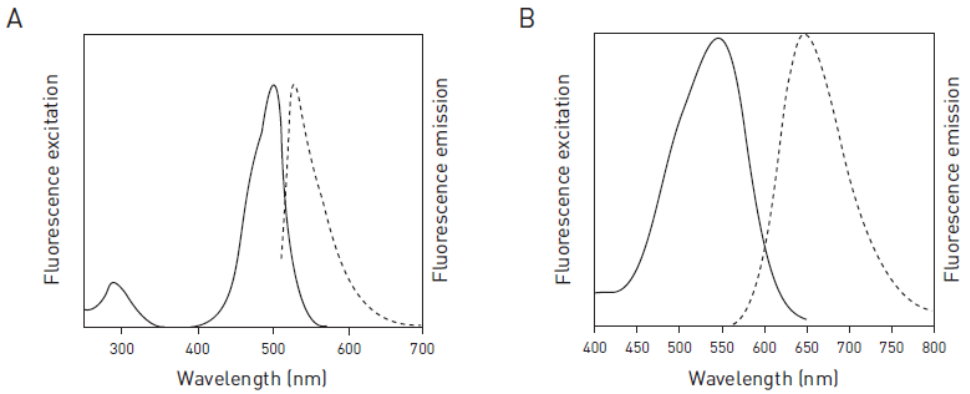

Figure 2 Spectra for the CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit.
Fluorescence excitation and emission spectra of the (A) CellEvent™ Caspase-3/7 Green Detection Reagent following cleavage of the DEVD peptide sequence and (B) SYTOX™ AADvanced™ Dead Cell Stain, bound to DNA.

Contents and storage

Component	Amount		Concentration	Storage[1]
	Cat. No. C10427	Cat. No. C10740		
CellEvent™ Caspase-3/7 Green Detection Reagent (Component A)	100 tests	20 tests	500 µM	≤–20°C Desiccate Store vial upright Protect from light
SYTOX™ AADvanced™ Dead Cell Stain (Component B)	100 tests	20 tests	1 mM[2]	
Dimethylsulfoxide (DMSO) (Component C)	200 µL	200 µL	Not applicable	
Number of reactions: Sufficient material is supplied for 100 reactions (Cat. No. C10427) or 20 reactions (Cat. No. C10740) based on the protocol "Stain cells with CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit" on page 2.				
Approximate fluorescence excitation and emission maxima (bound to DNA): CellEvent™ Caspase-3/7 Green Detection Reagent = approximately 511/533 nm; SYTOX™ AADvanced™ Dead Cell Stain = 546/647 nm (see Figure 2).				

[1] When stored as directed, kit components are stable for at least 1 year.
[2] When reconstituted with DMSO. Before refreezing, tightly seal the vial.

Before you begin

Required materials not supplied

- Cells of interest in single-cell suspension (appropriate sample concentrations range from 1×10^5 to 1×10^7 cells/mL)
- Appropriate suspension buffer (e.g., phosphate buffered saline, complete media, etc.)
- *(Optional)* Inducing agent appropriate for the cell model used (e.g., camptothecin, staurosporine)
- *(Optional)* Control sample (no treatment)

Label cells for flow cytometry

1 Stain cells with CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit

The following procedure was developed using the Jurkat T-cell leukemia cell line but can be adapted for any cell type. Growth medium, cell density, cell type variations, and other factors may influence staining. In initial experiments, test a range of stain concentrations to determine the optimal stain concentration for the given cell type, buffer, and experimental conditions.

1 Stain cells with CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit *(continued)*

1.1. *(Optional)* Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of apoptosis inducing agent.

1.2. Harvest the cell sample(s). Adjust the cell concentration of the sample(s) between 1×10^5 cells/mL and 1×10^7 cells/mL using the appropriate buffer such as 1X PBS (Cat. No. 10010) ± 2% BSA, or complete growth medium (e.g., RPMI, Cat. No. 22400; DMEM, Cat. No. 11995).

1.3. Before using the SYTOX™ AADvanced™ Dead Cell Stain for the first time, prepare a 1 mM solution.

- **a.** Remove the vial containing the SYTOX™ AADvanced™ Dead Cell Stain (Component B) and the vial of DMSO (Component C) from the freezer and allow the contents to equilibrate to room temperature.
- **b.** Add 100 µL of DMSO to the vial of SYTOX™ AADvanced™ Dead Cell Stain and mix well.

1.4. Prepare flow cytometry tubes each containing 1 mL of cell suspension. We recommend that you prepare and acquire single stained compensation controls using the CellEvent™ Caspase-3/7 Green Detection Reagent and the SYTOX™ AADvanced™ Dead Cell Stain.

1.5. Add 1 µL of CellEvent™ Caspase-3/7 Green Detection Reagent to 1 mL of sample and mix gently. Incubate the samples for 30 minutes at 37°C or 45–60 minutes at room temperature, protected from light. The final concentration of the reagent is 500 nM.

Note: For other cell types and models, stain concentration and labeling duration may require adjustment. For optimization, we recommend testing final concentrations between 400–1,000 nM.

1.6. During the final 5 minutes of staining, add 1 µL of the 1 mM SYTOX™ AADvanced™ Dead Cell Stain in DMSO to the appropriate samples and mix gently. The final labeling concentration of stain is 1 µM.

1.7. Analyze the samples without washing or fixing, using 488-nm excitation and collecting fluorescence emission using a 530/30 bandpass filter or equivalent for CellEvent™ Caspase-3/7 Green Detection Reagent and a 690/50 bandpass filter or equivalent for SYTOX™ AADvanced™ Dead Cell Stain.

Note: Alternative laser light sources (e.g., 532 nm and 561 nm) are also compatible with the SYTOX™ AADvanced™ Dead Cell Stain.

1.8. Following application of standard fluorescence compensation technique, 3 cell populations should be visible on a dual parameter dot plot of CellEvent™ Caspase-3/7 Green Detection Reagent fluorescence versus SYTOX™ AADvanced™ Dead Cell Stain fluorescence (see Figure 1).

2 Perform multicolor staining

CellEvent™ Caspase-3/7 Green Detection Reagent and the SYTOX™ AADvanced™ Dead Cell Stain have minimal spectral overlap with fluorophores excited by other laser lines, and they can be combined with other dyes excitable by the 488-nm laser or other lasers. If used in combination with other reagents for multicolor applications, apply the other dyes to the sample first following manufacturer's instructions, and then apply the SYTOX™ AADvanced™ Dead Cell Stain as the last stain to the sample. Do not wash or fix samples prior to flow cytometric analysis.

Related products

Product	Cat. No.	Unit size
CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit	C10427	100 assays
CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit	C10740	20 assays
CellEvent™ Caspase-3/7 Green Detection Reagent	C10423	100 µL
CellEvent™ Caspase-3/7 Green Detection Reagent	C10723	20 µL

Life Technologies Corporation | 29851 Willow Creek Road | Eugene, Oregon 97402 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0007522

Revision	Date	Description
B.0	29 July 2022	The format and content were updated.
A.0	15 June 2016	Information on Cat. No. C10740 was added.
1.0	29 November 2012	New document for the CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit.

The information in this guide is subject to change without notice.