molecular probes®
by life technologies™

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

Catalog no. C10427

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
CellEvent® Caspase 3/7 Green Detection Reagent (Component A)	100 µL	500 µM	• ≤–20°C • Desiccate • Store vial upright • Protect from light	When stored as directed, kit components are stable for at least 1 year.
SYTOX® AADvanced™ Dead Cell Stain (Component B)	1 vial	1mM*		
Dimethylsulfoxide (DMSO, Component C)	100 µL	N/A		
*When reconstituted with DMSO. Before refreezing, seal the vial tightly.				
Number of reactions: Sufficient material is supplied for 100 reactions, based on the protocol below.				
Approximate fluorescence excitation and emission maxima (bound to DNA): CellEvent® Caspase 3/7 Green Detection Reagent ~511/533 nm; SYTOX® AADvanced™ dead cell stain 546/647 nm (see Figure 1, page 2).				

Introduction

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 that are triggered in response to pro-apoptotic signals and result in the cleavage of protein substrates and in the subsequent disassembly of the cell.[1] The recognition sequence in the target substrate always includes an aspartic acid residue; cleavage takes place at the carbonyl end of that residue.[2]

CellEvent® Caspase-3/7 Green Detection Reagent is a novel fluorogenic substrate for detection of activated caspases 3 and 7 in apoptotic cells. This cell-permeant reagent consists of a four 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 (Figure 2, page 2).

Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. Life Technologies offers a wide selection of products for apoptosis research; for more information, refer to www.lifetechnologies.com/flowcytometry.

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 1 (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 1 Fluorescence excitation and emission spectra of the (A) the CellEvent® Caspase 3/7 Green Detection Reagent following cleavage of the DEVD peptide sequence and (B) SYTOX® AADvanced™ dead cell stain, bound to DNA.

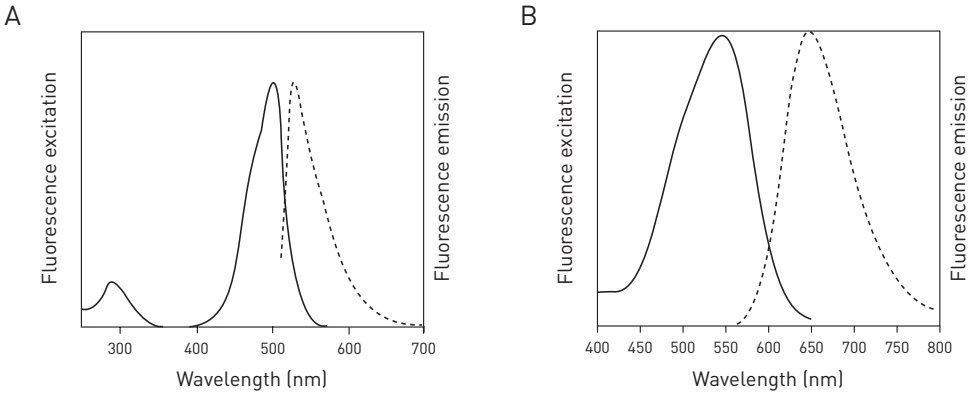
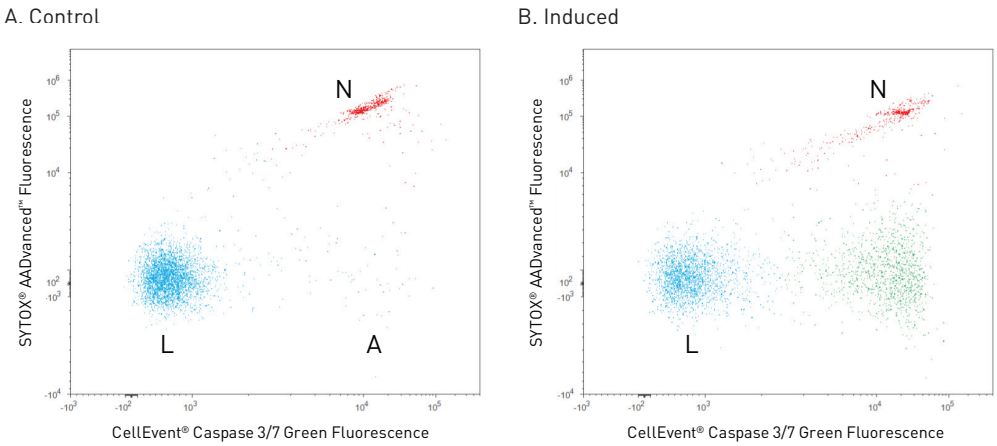


Figure 2 Jurkat cells (T-cell leukemia, human) were treated with (A) DMSO or (B) 10 µM camptothecin for 3 hours before labeling with the CellEvent® Caspase 3/7 Green Flow Cytometry kit. Stained samples were analyzed on the Attune® Acoustic Focusing Cytometer equipped with a 488-nm laser, and fluorescence emission was collected using a 530/30 BP filter for CellEvent® Caspase 3/7 Green Detection Reagent and a 690/50BP filter for SYTOX® AADvanced™ 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.



Before You Begin

Materials Required but not Provided

- Cells of interest in single cell suspension (appropriate sample concentrations range from 1×10^5–1×10^7 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)

Caution

No data are available addressing the mutagenicity or toxicity of the reagents within this kit. Because both Components A and B bind to nucleic acids, they should be treated as potential mutagens and used with appropriate care.

The DMSO stock solution should be handled with particular caution, because 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 with plenty of water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling these reagents.

Storage and Handling

Upon receipt, the contents of this kit should be stored frozen at –20°C, upright, desiccated, and protected from light. Before refreezing, seal the vials tightly. When stored properly, the stock solutions are stable for at least one year. This kit contains sufficient material to assay 100 samples using the method outlined below.

Experimental Protocols

Staining Protocol

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.

1.1 *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 400–1000 nM.

1.6 During the final 5 minutes of staining, add 1 µL of the 1 mM SYTOX® AADvanced™ dead cell stain solution 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 BP filter or equivalent for SYTOX® AADvanced™ dead cell stain.

Note: Alternative laser light sources such as 532-nm and 561-nm are also compatible with the SYTOX® AADvanced™ dead cell stain.

1.8 Following application of standard fluorescence compensation technique, three cell populations should be visible on a dual parameter dot plot of CellEvent® Caspase 3/7 Detection Reagent fluorescence versus SYTOX® AADvanced™ fluorescence (see Figure 2, page 2).

Multicolor Staining

CellEvent® Caspase 3/7 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™ stain as the last stain to the sample. Do not wash or fix samples prior to flow cytometric analysis.

Reference

1. Cell Death and Diff. 6, 1067 (1999); **2.** J.Biol. Chem. 272, 17907 (1997).

Product List

Current prices may be obtained from www.lifetechnologies.com or from our Customer Service Department.

Catalog no.	Product Name	Unit Size
C10427	CellEvent® Caspase-3/7 Green Flow Cytometry Assay Kit *100 assays*	1 kit
Related Products		
C10423	CellEvent® Caspase-3/7 Green Detection Reagent *2 mM solution in DMSO*	100 µL
A23204	annexin V, Alexa Fluor® 647 conjugate *100 assays*	500 µL
A13201	annexin V, Alexa Fluor® 488 conjugate *100 assays*	500 µL
A23210	APO-BrdU™ Alexa Fluor® 488 TUNEL Assay Kit *60 assays*	500 µL
A13199	annexin V, fluorescein conjugate (FITC annexin V) *100 assays*	500 µL
A35108	annexin V, Alexa Fluor® 555 conjugate *100 assays*	500 µL
A35110	annexin V, R-phycoerythrin conjugate (R-PE annexin V) *50 assays*	250 µL
V13246	Annexin-binding buffer *5X concentrate* *for flow cytometry*	50 mL
V13244	Chromatin Condensation/Dead Cell Apoptosis Kit *Hoechst 33342/propidium iodide* *200 assays* *for flow cytometry*	1 kit
V23200	Vybrant® Apoptosis Assay Kit #6 *biotin-X annexin V/Alexa Fluor® 350 streptavidin/propidium iodide* *50 assays*	1 kit
V35123	Violet Membrane Permeability/Dead Cell Apoptosis Kit *with PO-PRO®-1 and 7- aminoactinomycin D* *200 assays* *for flow cytometry*	1 kit
V35136	Violet Annexin V/Dead Cell Apoptosis Kit *Pacific Blue™ annexin V/SYTOX® AADvanced™* *for flow cytometry* *50 assays*	1 kit
V35112	PE Annexin V/ Dead Cell Apoptosis Kit *with SYTOX® Green* *50 assays* *for flow cytometry*	1 kit
V35113	APC Annexin V/Dead Cell Apoptosis Kit *with APC annexin V and SYTOX® Green* *50 assays* *for flow cytometry*	1 kit
S10274	SYTOX® AADvanced™ dead cell stain *for 488 excitation* *for flow cytometry* *500 tests*	1 kit
S10349	SYTOX® AADvanced™ dead cell stain *for 488 excitation* *for flow cytometry* *100 tests*	1 kit
S34857	SYTOX® Blue dead cell stain *for flow cytometry* *1000 assays* *1 mM solution in DMSO*	1 mL
S34861	SYTOX® Orange dead cell stain *for flow cytometry* *250 µM* *1000 tests*	1 mL
S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL
S34862	SYTOX® Dead Cell Stain Sampler Kit *for flow cytometry* *50 tests per vial*	1 kit
L34960	LIVE/DEAD® Fixable Dead Cell Stain Sampler Kit *for flow cytometry* *320 assays*	1 kit
V13243	Membrane Permeability/ Dead Cell Apoptosis Kit *YO-PRO®-1/propidium iodide* *200 assays* *for flow cytometry*	1 kit
A35137	Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit *for flow cytometry* *100 assays*	1 kit

Purchaser Notification

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European Headquarters
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese Headquarters
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
Fax: +81 3 5730 6519
Email: jpinfo@invitrogen.com

Additional international offices are listed at www.lifetechnologies.com

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 www.lifetechnologies.com.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- 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 www.lifetechnologies.com/sds.

Certificate of Analysis
The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

For Research Use Only. Not for use in diagnostic procedures.

Disclaimer
LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Limited Product Warranty
Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Limited Use Label License: Research Use Only
The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

life technologies™