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

Catalog Number C10747, C10748

Pub. No. MAN0016085 **Rev.** A.0

Table 1. Contents and storage

Material	Amount		Concentration	Storage*
	C10747	C10748		
CellEvent™ Caspase 3/7 Red Detection Reagent (Component A)	20 tests	100 tests	1.25 mM	• ≤–20°C • Desiccate • Store vial upright • Protect from light
* When stored as directed, kit components are stable for at least 1 year.				
Number of reactions: Sufficient material is supplied for 20 reactions (Cat. No. C10747) or 100 reactions (Cat. No. C10748), based on the protocol below.				
Approximate fluorescence excitation and emission maxima (bound to DNA): CellEvent™ Caspase 3/7 Red Detection Reagent ~630/650 nm.				

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 Red 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™ Green Dead Cell Stain (Cat. No. S34860), apoptotic cells can easily be discriminated from live and 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 **www.thermofisher.com/flowcytometry**.

Spectral characteristics

The absorption and fluorescence emission maxima of the CellEvent™ Red Reagent/DNA and SYTOX™ Green stain/DNA complexes are 630 nm/650 nm and 504 nm/523 nm, respectively. The CellEvent™ Caspase 3/7 Red Detection Reagent exhibits greater than a 30-fold increase upon cleavage and binding DNA. Similarly, the SYTOX™ Green Dead Cell Stain exhibits a fluorescence enhancement of greater than 100-fold.

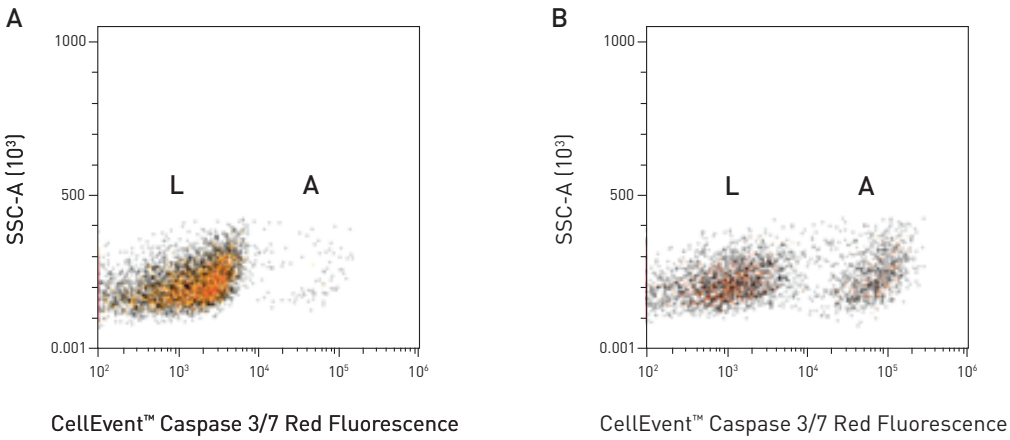

Figure 1. Caspase activity detection in Jurkat cells using the CellEvent™ Caspase 3/7 Red Flow Cytometry Assay Kit. Jurkat cells were treated with **(A)** DMSO or **(B)** 0.02 µM staurosporine for 4 hours before labeling with CellEvent™ Caspase 3/7 Red Flow Cytometry Assay kit. Stained samples were analyzed on an Attune™ NxT Acoustic Focusing Cytometer equipped with a 637-nm laser, and fluorescence emission was collected using a 670/14BP filter. Treated cells have a higher percentage of apoptotic cells **(B)** than the basal levels displayed by the control **(A).** A = apoptotic cells, L = viable cells.

Before you begin

Materials required but not supplied

- 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:* SYTOX™ Green Dead Cell Stain (Cat. No. S34860)
- *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, store the kit contents 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. The kits contain sufficient material to assay 20 samples (Cat. No. C10747) or 100 samples (Cat. No. C10748) using the method outlined below.

Methods

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 samples. 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 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 Red Detection Reagent.

Note: If you are using the optional SYTOX™ Green Dead Cell Stain, prepare and acquire single stained compensation controls using this reagent as well.

1.4 Add 1 µL of CellEvent™ Caspase-3/7 Red 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 1.25 µM.

Note: For other cell types and models, stain concentration and labeling duration may require adjustment. For optimization, we recommend testing final concentrations between 400 nM–2.5 µM.

1.5 *Optional:* During the final 5 minutes of staining, add 1 µL of the 30 µM SYTOX™ Green Dead Cell Stain solution in DMSO to the appropriate samples and mix gently. The final labeling concentration of the SYTOX™ Green stain is 30 nM.

1.7 Analyze the samples without washing or fixing. For the CellEvent™ Caspase-3/7 Red Detection Reagent, use the appropriate instrument filter sets for 630 nm excitation and 650 nm emission. We recommend using an Alexa Fluor™ 647/Cy®5 filter.

Note: For the optional SYTOX™ Green Dead Cell Stain, use 488-nm laser for excitation and collect fluorescence emission using a 530/30 bandpass filter or equivalent.

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 Red Detection Reagent fluorescence versus SYTOX™ Green fluorescence.

Multicolor staining

CellEvent™ Caspase 3/7 Red Detection Reagent and the SYTOX™ Green 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™ Green stain as the last stain to the sample. Do not wash or fix samples prior to flow cytometric analysis.

References

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

Product list

Cat. No.	Product name	Unit size
C10747	CellEvent™ Caspase-3/7 Red Flow Cytometry Assay Kit *20 assays*	1 kit
C10748	CellEvent™ Caspase-3/7 Red Flow Cytometry Assay Kit *100 assays*	1 kit
Related products		
C10427	CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit *100 assays*	1 kit
C10740	CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit *20 assays*	1 kit
C10423	CellEvent™ Caspase-3/7 Green Detection Reagent *5 mM solution in DMSO*	100 µL
C10723	CellEvent™ Caspase-3/7 Green Detection Reagent *5 mM solution in DMSO*	20 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™ Green* *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™ Green Dead Cell Stain *for 488 excitation* *for flow cytometry* *500 tests*	1 kit
S10349	SYTOX™ Green 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
S34860	SYTOX™ Green dead cell stain *for flow cytometry* *30 µM* *1000 tests*	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

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
- Search through frequently asked questions (FAQs)
- 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**.

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 **thermofisher.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

Limited Product Warranty
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Revision	Date	Description
A.0	August 2016	New document

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