

SR-VAD-OPH

in vitro Apoptosis Detection Reagent

Catalog #6357

FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures.

INTRODUCTION

Apoptosis is an evolutionarily conserved process of programmed cell suicide that centers on a cascade of proteolytic enzymes called caspases that are triggered in response to pro-apoptotic signals. Once activated, caspases cleave protein substrates leading to the eventual disassembly of the cell¹.

Early research studies using short peptide sequences linked to an aldehyde or fluoromethyl ketone (FMK) reactive group identified a number of peptide sequences preferentially targeted by different caspase enzymes. For example, the sequence Asp-Glu-Val-Asp (DEVD) is cleaved by caspase-3 and the sequence Val-Ala-Asp (VAD) demonstrates multiple caspase (caspase -1, -2, -3, -4, -5, -6, -7 -8, -9) recognition properties².

ImmunoChemistry Technologies utilized these findings to develop the often-cited FLICA® (Fluorescence Labeled Inhibitors of CASpases) caspase inhibitor reagents, which have been widely used for years as simple and reliable methods for screening apoptosis in live cells and tissues. These reagents were made by sandwiching target peptide sequences with varying caspase specificities between a red fluorescent label, sulforhodamine B (SR), and an FMK reactive moiety. The resulting red fluorescent, cell-permeant, non-cytotoxic inhibitor reagents can be added to cell culture media for *in vitro* use. They will cross the cell membrane and form irreversible covalent bonds with activated caspase enzymes present inside apoptotic cells, which can then be differentiated from non-apoptotic cells by their retained red fluorescence.

ImmunoChemistry Technologies has now released a novel set of inhibitor reagents that employ an O-phenoxy (OPH) reactive group instead of an FMK group. In a manner analogous to the FMK class of cysteine reactive compounds, the OPH inhibitors form a stable covalent thioether adduct with the reactive SH-site of caspase enzymes present in apoptotic cells³. OPH inhibitor compounds have the benefit of being extremely non-cytotoxic, display enhanced stability characteristics compared to the FMK analogs, and have been optimized to provide a high level of performance for *in vitro* applications³⁻⁴.

REAGENT

- SR-VAD-OPH (SR-Val-Ala-Asp(OMe)-Oph (2,6-diF)) poly caspase inhibitor
#6357 (4/pack); MW = 970.2

DETECTION EQUIPMENT

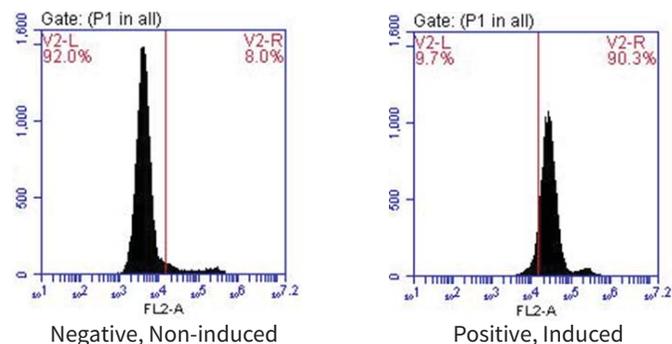
SR-VAD-OPH excites at 565 nm and emits at 590-600 nm. Use laser/filter pairings that best approximate these settings. The fluorescent signal may be analyzed by:

- Fluorescence microscope
- Fluorescence plate reader
- Flow cytometer



FIGURE 1: SINGLE COLOR ANALYSIS VIA FLOW CYTOMETRY

Jurkat cells (T lymphocytes) were treated with 1 μ M staurosporine for 4 hours to induce apoptosis via caspase activity (Positive, Induced, right histogram). Negative control cells were spiked with a DMSO vehicle control (Negative, Non-induced, left histogram). Cells were then stained with ICT's red poly caspase inhibitor probe, SR-VAD-OPH for 1 hour, washed twice, and read on an Accuri C6 flow cytometer. Treatment with the negative control induced caspase activity in only 8.0% of the cell population, whereas treatment with staurosporine induced caspase activity in 90.3% of the experimental cells. This is a ratio of 11:1. Data courtesy of Ms. Tracy Hanson, ICT 092713.



STORAGE

Store unopened reagents at $\leq 2-8^{\circ}\text{C}$ until the expiration date. Once reconstituted in DMSO, use immediately, or store at $\leq -20^{\circ}\text{C}$ for 6 months protected from light and thawed no more than twice during that time.

SAFETY

- See Safety Data Sheet (SDS) for any warnings.
- SDS are available at online at www.immunochemistry.com or by calling 1-800-829-3194 or 952-888-8788.
- For research use only.
- Not for use in diagnostic procedures.



HOW TO USE

1. Prepare experimental samples and control cell populations. Ideally, cell concentration should be $3\text{-}5 \times 10^5$ cells/mL. The concentration should not exceed 10^6 cells/mL, as cells cultivated in excess of this concentration may begin to naturally enter apoptosis. Just prior to staining, cells may need to be concentrated to $2\text{-}5 \times 10^6$ cells/mL as both microscopy and plate reader analysis methods require higher cell concentrations than flow cytometry. Start with a larger volume of cells at $3\text{-}5 \times 10^5$ cells/mL (which is a typical density for cell culture) and then concentrate cells and resuspend to 290-300 μL per sample when ready to label.
2. Reconstitute SR-VAD-OPH with 50 μL DMSO to form the 150X stock concentrate.
3. Dilute the 150X stock concentrate 1:5 by adding 200 μL PBS to create the 30X working solution.
4. Add 10 μL of the 30X working solution to each sample (~290 μL aliquot of cultured cells). For different volumes, add the reagent at 1:30. Some cells may require more or less reagent; perform an initial titration experiment to determine the ideal concentration.
5. Incubate approximately 1 hour.
6. Wash cells to remove any unbound reagent:
 - a. Add 1-2 mL PBS or cell culture media and centrifuge at 200 x g for 5-10 minutes at room temperature (RT).
 - b. Carefully remove and discard supernatants.
 - c. Resuspend in 1-2 mL PBS or cell culture media and centrifuge at 200 x g for 5-10 minutes at RT.
 - d. Carefully remove and discard supernatants.
 - e. Gently vortex pellets to disrupt clumping.
 - f. Resuspend cells in 300 μL PBS or cell culture media and gently mix.
7. If desired, label with additional stains, such as Hoechst, DAPI, or an antibody. Wash cells if necessary to remove excess stain from the media.
8. If desired, fix or mount cells.
9. Analyze with a fluorescence microscope, plate reader, or flow cytometer. SR-VAD-OPH excites at 565 nm and emits at 590-600 nm.
10. For more information, contact ICT at 1-800-829-3194 or visit www.immunochemistry.com.

REFERENCES

1. Slee, E. A., Adrain, C. & Martin, S. J. Serial killers: ordering caspase activation events in apoptosis. *Cell Death Differ* 6, 1067-1074, doi:10.1038/sj.cdd.4400601 (1999).
2. Thornberry, N. A., et. al. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. *J Biol Chem* 272, 17907-17911 (1997).
3. Chauvier, D., Ankri, S., Charriaut-Marlanque C., Casimir R. & Jacotot E. Broad spectrum caspase inhibitors: from myth to reality?. *Cell Death Differ* 14, 387-391, doi: 10.1038/sj.cdd.4402044 (2007).
4. Callus, B. A. & Vaux, D. L. Caspase inhibitors: viral, cellular, and chemical. *Cell Death Differ* 14:73-78 doi:10.1038/sj.cdd.4402034 (2007).

FIGURE 2: FLUORESCENCE MICROSCOPY IMAGING

Jurkat cells (T lymphocytes) were treated with 1 μM staurosporine for 3 hours at 37°C to induce apoptosis. Cells were then labeled with ICT's red fluorescent poly caspase inhibitor SR-VAD-OPH for 60 minutes. Cells were washed twice and wet-mount slides were prepared. A grey differential interference contrast (DIC) image (left) was taken, and red fluorescence (right) was detected using a band pass filter (excitation at 565 nm, emission at 590-600 nm). SR-VAD-OPH reveals complete caspase activation in the form of red fluorescence in all cells. Data courtesy of Ms. Tracy Hanson, ICT (ICT111809; SR_OPH_111_17_18).

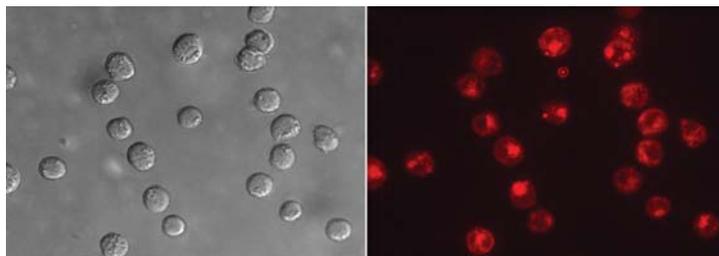
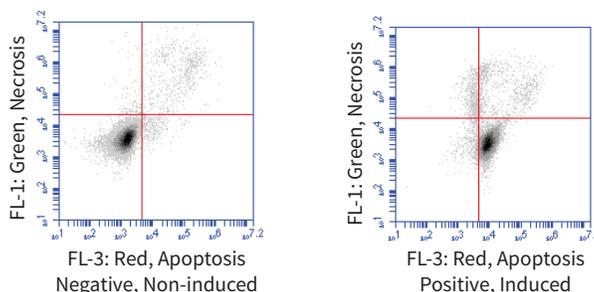


FIGURE 3: USE SR-VAD-OPH WITH GREEN LIVE/DEAD STAIN TO QUANTITATE 4 POPULATIONS OF APOPTOSIS AND NECROSIS WITHIN A SINGLE SAMPLE

ICT's red fluorescent poly caspase inhibitor reagent, SR-VAD-OPH, was used with Green Live/Dead Stain (catalog #6342), a green vital stain, to assess apoptosis and necrosis in Jurkat cells. Jurkat cells were treated with DMSO as a control (left) or 1 μM staurosporine, an apoptosis inducer (right), for 4 hours at 37°C. Cells were then labeled with SR-VAD-OPH for 1 hour, washed, and dually stained with the Green Live/Dead Stain. Cells were analyzed using two-color flow cytometry with an Accuri C6. Density plots were set up to detect caspase activity (red, FL-3) on the X-axis and necrosis (green, FL-1) on the Y-axis.

Four populations of cells were detected: unstained live cells (lower left); cells in early apoptosis fluoresce red with SR-VAD-OPH (lower right); cells in late apoptosis (upper right) are dually stained with SR-VAD-OPH and Green Live/Dead Stain they fluoresce red (they have active caspases) and green (the cell membrane has permeabilized); and necrotic cells fluoresce green (upper left). Very few cells were killed when treated with the placebo (left plot), while many cells entered apoptosis and secondary necrosis when treated with staurosporine (right plot). Data courtesy of Tracy Hanson, ICT (ICT-202:97; 091015).



Thank you for using SR-VAD-OPH!

If you have any questions, or would like to share your data, please contact us at help@immunochemistry.com.



BRIGHT MINDS, BRIGHT SOLUTIONS.

ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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