

SR-FLICA Poly Caspase Assay Kit | SR-FLICA assay kit

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CATALOG #
AAA52714

PRODUCT SYNONYMS

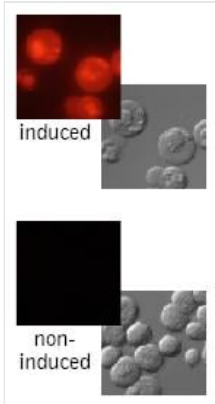
SR-FLICA Poly Caspase; SR-FLICA Poly Caspase Assay Kit; SR-FLICA assay kit

PRODUCT SPECIFICATIONS

Reagents	SR-VAD-FMK
Suspension Cells	Culture your cells up to 1×10^6 cells/mL. Follow experimental protocol where caspase activity will be investigated; create positive and negative controls for caspase activity. Reconstitute the reagent with 50uL DMSO to form the stock concentrate (can be frozen for future use). Dilute the stock concentrate with 200uL 1X PBS to form the working solution. Add ~10uL of the working solution directly to a 300-500uL aliquot of your cell culture for labeling. Incubate 30 minutes -1 hour. Wash and spin cells two or three times, or let incubate for 1 hour with fresh media or 1x apoptosis wash buffer. If desired, label cells with Hoechst stain. If desired, fix cells. Analyze data using a fluorescence microscope or fluorescence plate reader.
Frozen Tissue	Prepare tissue sections according to the experiment. Do not paraffin-embed or fix. Allow frozen sections to thaw before FLICA labeling. Reconstitute lyophilized FLICA reagent with 50 uL DMSO to form a stock concentrate. Dilute 10X Apoptosis Wash Buffer (in kit) to 1X. Just prior to staining the samples, dilute the stock concentrate 1:50 in PBS to form the FLICA tissue staining working solution. Add enough FLICA working solution to cover the sample. Incubate at least 1 hour, protected from light. Wash with TBSt, PBSt, or 1X Apoptosis Wash Buffer twice for 5-10 minutes. Place slides in incubation dish containing 1X Apoptosis Wash Buffer Stain nuclei with DAPI and apply coverslip. Store samples at 2-8 degree C for short term storage or at -20 degree C for long-term storage.
Adherent Cells	Adherent cells need to be carefully washed to avoid the loss of any cells which round up and come off the plate surface. Loose cells may be harvested from the plate or slide surface and treated as suspension cells, while those remaining adherent to the surface should be washed as adherent cells. If the adherent cells are trypsinized, the loose cells can be recombined with the trypsinized pool, or the washed loose cells can then be recombined with the adherent portion when the analysis is performed. If growing adherent cells on a tissue culture plate, the entire plate may be gently spun as part of the wash process to sediment any loose floating cells. Avoid any attempts to trypsinize cells prior to labeling with a vital dye such as PI. Trypsin exposed cell membranes could become transiently permeant to vital dyes for a variable time period, depending upon the cell line. Cells may be labeled with FLICA before or after trypsinization.
Adherent Cells: Trypsinization prior to FLICA labeling and FACS analysis	Culture cells in T25 flasks and expose to the experimental conditions. Apoptotic cells may detach and begin to float into the media. Save and spin to pellet and include these cells in your analysis. Trypsinize adherent cells; neutralize with trypsin inhibitor present in 20% FBS-cell culture media; pool cells with any pellets created in #2; add a few mL media. Spin ~5 minutes at 220 x g and remove all but ~100 uL supernatant. Count cells and adjust volume of cell suspension to fit the experiment (typically 300-500 uL). Transfer cells into a 15 mL tube. Add 10 - 17 uL of 30X FLICA. Incubate at 37 degree C, 30-60 minutes, mixing gently every 10 minutes. Wash by adding ~10mL media and incubate at 37 degree C for 60 minutes to allow any unbound FLICA to diffuse out of the cells. Spin at 220 x g for 5 minutes; aspirate supernatant. Add ~300uL 1X apoptosis wash buffer. Put cells on ice, and protect from light. If desired, add 30 uL fixative. Analyze cells via fluorescence microscopy, fluorescence plate reader, or flow cytometry (yellow or green excitation laser required for appropriate excitation of the sulforhodamine B dye label).

Adherent Cells: FLICA label prior to trypsinizing, and FACS analysis	Seed 5-8 x 10 ⁴ cells in a 24-well plate in a final volume of 600 uL and let attach for 24 hours. Expose cells to the experimental conditions. Add 1-4 uL of FLICA 150X stock concentrate and incubate 1-3 hours at 37 degree C. Remove supernatant containing any rounded up cells and set aside in labeled tube. Wash adherent cell monolayer by gently adding PBS to cover the adherent cell monolayer. Remove PBS and combine with cells previously set aside in step 4. Add trypsin - versene to barely cover the attached cell monolayer. Allow cells to detach and remove detached cells by adding 1 mL of cell culture media + 20% FBS to the trypsinized cells in the wells. Add detached cells from the trypsinization step to supernatant from step 4. Add 2 mL of cell culture media + 20% FBS to each tube containing trypsinized cells. Spin cells at 220 x g for 5 min. Remove supernatant and discard. Add 1mL 1x apoptosis wash buffer. Spin cells at 220 x g for 5 min. Remove supernatant. Add 1mL 1x apoptosis wash buffer. Spin cells at 220 x g for 5 min. Remove supernatant and resuspend in 300 uL 1X apoptosis wash buffer. If desired, add 30uL fixative. Analyze cells via fluorescence microscopy, fluorescence plate reader, or flow cytometry (yellow or green excitation laser required for appropriate excitation of the sulforhodamine B dye label).
Target	Poly Caspases
Excitation / Emission	565 nm / 586 nm
Method of Analysis	Flow Cytometer, Fluorescence Microscope, Fluorescence Plate Reader
Types of Samples	Cell Culture, Tissue
Preparation and Storage	Store at 2-8 degree C

IMAGES



Application Data
Jurkat cells were treated with 1uM staurosporine to induce caspaseactivity (top), or a negative control (bottom) for 3 hours, incubatedwith ICT's red poly caspases inhibitor probe, SR-VAD-FMK, for 1 hour,washed twice, and examined under a fluorescence microscope (DICimages were also taken). The color image of induced cells (upper left)reveals experimental cells which fluoresce red, therefore they all havesome degree of caspase activity. The non-induced DIC image (lowerright) reveals many control cells, however the correspondingfluorescence image (lower left) is dark; none of these cells have activecaspases (Dr. Brian W. Lee, ICT).

ADDITIONAL INFORMATION

Related Product Information for SR-FLICA assay kit	Description: Assay for apoptosis via poly caspase activation with the red SR FLICA Poly Caspase Assay Kit. This in vitro, whole cell caspase assay employs the red fluorescent caspase inhibitor probe SR-VAD-FMK to label activated caspase enzymes in living cells or tissue samples. Analyze the fluorescent signal in whole cells using fluorescence microscopy, a fluorescent plate reader, or by flow cytometry (green or yellow laser required to detect SR). Methodology: FLICA caspase and apoptosis detection reagents are comprised of an inhibitor peptide sequence that binds to activated caspase enzymes, a fluoromethyl ketone (FMK) moiety that results in an irreversible binding event with the enzyme, and a fluorescent tag reporter. For a poly-caspase inhibitor, the multi-enzyme peptide recognition sequence is valine-alanine-aspartic acid (VAD). The FLICA probe interacts with the enzymatic reactive center of an activated caspase via the peptide recognition sequence, forming a covalent thioether adduct with the enzyme through the FMK moiety. FLICA probes are cell permeant and non- cytotoxic. After incubation with the cell sample, unbound SR-FLICA caspase detection reagent is washed away from non-apoptotic cells; the remaining red fluorescent signal is a direct measure of caspase activity at the time the probe was added. SR-FLICA is labeled with sulforhodamine B, a red fluorescent dye with optimal excitation at 565 nm and emission at 590- 600 nm. Detection of nuclear morphology is also possible with the additional kit component, Hoechst 33342. Background: SR-FLICA Poly Caspase Assay for Apoptosis Detection in Whole Cell and Tissue Samples Apoptosis is an evolutionarily conserved form of cell suicide mediated by a cascade of proteolytic enzymes called caspases. Pro-apoptotic signals activate the enzymatic cascade resulting in the cleavage of protein substrates, leading to the disassembly of the cell (1-4). Caspases have been identified in organisms ranging from C. elegans to humans. Members of the mammalian caspase family of cysteinyl aspartate-specific proteases play distinct roles in apoptosis and inflammation. Caspases: Cysteine Proteases There are two types of caspases; the initiators (caspases 8, 9, and 10) and the effector caspases (caspases 1, 2, 3, 4, 6, 7, 12, and 13). The initiator caspases 8 and 10 are also referred to as the extrinsic apoptosis pathway that originates upon activation of cell surface death receptors. Caspases 8 and 10 are monomers that bind to death receptor proteins through their death effector domain (DED) structure. Caspase 9 is also called the intrinsic pathway that results from the mitochondrial release of cytochrome c. The initiator caspase 9 monomer binds other proteins through their caspase activation and recruitment domain (CARD). The initiator caspase -protein interaction results in dimerization of the initiator caspases that leads to their activation. These activated initiator caspases then cleave the effector pro-caspases at specific aspartic acid residues to yield large (20 kDa) and small (10 kDa) subunits that then assemble into the heterotetrameric, catalytically active form of the caspase effector enzymes (5, 6). Active caspase enzymes exhibit catalytic and substrate specificities comprised of short tetra-peptide amino acid sequences that must contain an aspartate in the P1 position (7 - 9). These preferred tetra-peptide sequences have been used to derive peptides that specifically compete for caspase binding (4 - 6). In addition to the distinctive aspartate cleavage site at P1, the catalytic domains of the caspases require typically four amino acids to the left of the cleavage site with P4 as the prominent specificity-determining residue (9). In contrast to this tetrapeptide specificity, the tri-peptide VAD is able to bind to the active site of every caspase family member studied. Furthermore, addition of a fluoromethyl ketone (FMK) to the tri-peptide results in an irreversible linkage and permanent inactivation of the cysteine protease enzyme (10). Accordingly, the Z-VAD-FMK inhibitor has been shown in numerous studies to effectively inhibit the induction of apoptosis by blocking caspase activation (9, 11). Furthermore, substitution of the amino terminal benzyloxycarbonyl blocking group (Z-) with a detection moiety, such as a fluorescent dye, yields a probe that allows for the detection of caspase activity (12 - 14). FLICA: Fluorescent-Labeled, Inhibitor-Based Caspase Assays The FLICA methodology of caspase detection is available in kit form for assessing individual or poly-caspase activity in cultured cells and tissues. Upon addition to a cultured cell sample, the cell-permeant poly-caspase FLICA SR-VAD-FMK reagent will enter each cell and form irreversible bonds with activated intracellular caspases. Because FLICA reagent becomes covalently coupled to the active enzyme, it is retained within the cell during wash steps, while any unbound FLICA reagent diffuses out of the cell and is washed away. The remaining red fluorescent signal is a direct measure of the amount of caspase activity present in the cell at the time the reagent was added. Cells that contain the covalently bound FLICA SR-VAD-FMK poly caspase reagent can be analyzed by 96-well-plate based fluorometry, fluorescence microscopy, or flow cytometry (when a green laser is available). The sulforhodamine (SR) FLICA reagents have an optimal excitation range of 560 - 570 nm, and emission range from 590 - 600 nm. Cells labeled with FLICA may be read immediately or preserved for 24 hours using the Fixative included in the kit. Unfixed samples may be subsequently analyzed with Hoechst stain or 7-AAD to detect changes in nuclear morphology or necrosis, respectively. Other FLICA Caspase Assay Kits, containing the preferred caspase recognition amino acid sequences for caspases 1, 2, 3, 6, 8, 9, 10, and 13, are also available with green, orange-red, and far-red fluorescence.
Product Categories/Family for SR-FLICA assay kit	Apoptosis Assays/Apoptosis Detection

References

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