

FAM-FLICA Caspase 9 Assay Kit | FAM-FLICA assay kit

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CATALOG #
AAA52726

PRODUCT SYNONYMS

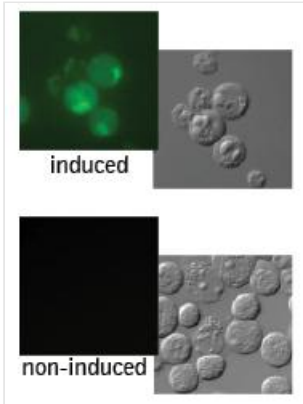
FAM-FLICA Caspase 9; FAM-FLICA Caspase 9 Assay Kit; FAM-FLICA assay kit

PRODUCT SPECIFICATIONS

Reagents	FAM-LEHD-FMK
Sample Protocol	FLICA, Fluorescent-Labeled Inhibitor of Caspases, is a simple yet accurate method to measure apoptosis via caspase activity in whole cells. Four sample protocols are outlined below.
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, label cells with Propidium Iodide or 7-AAD. If desired, fix cells. Analyze data using a fluorescence microscope, plate reader, or flow cytometer.
Frozen Tissue	Prepare frozen tissues according to the experiment. Allow slides to air-dry. Fix slides with acetone for 1 minute. Rehydrate slides by washing (twice for 5 min) in TBS-tween (TBSt) or PBS-tween (PBSt). Block slides for 20 minutes (such as 20% Aquablock in media with 0.2% tween). Dilute 150X FLICA stock 1:50 in PBS to form a 3X working solution. For example, add 50 uL 150X stock to 2450 uL PBS (2.5 mL total). Add 50 uL of 3X FLICA and incubate >1hr protected from light. Wash with TBSt or PBSt (twice for 5 min) by setting slides in slide incubation dish containing 1X wash buffer. Develop with DAPI and coverslip. Store samples at 2-8 degree C for short term storage, staining will last at -20 degree C for long periods.
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 with a flow cytometer.

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 on FACS immediately.
Target	Caspase 9
Excitation / Emission	488 nm / 530 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 1 uM staurosporine for 3 hours to induce caspase 9 activity (top), or were treated with a control (bottom). Both populations were incubated with ICT's green FAM-LEHD-FMK FLICA caspase 9 reagent. DIC images were taken of both samples. Almost all cells in the induced sample (top) fluoresce green, therefore they have activated caspase 9. None of the control cells (bottom) fluoresce green, therefore they do not have activated caspase 9 (Dr. Brian Lee, ICT).

ADDITIONAL INFORMATION

Related Product Information for FAM-FLICA assay kit	Description: Detect active caspase 9 with the green fluorescence FLICA Caspase 9 Assay Kit. This in vitro assay employs the fluorescent inhibitor probe FAM-LEHD-FMK to label active caspase 9 enzymes in living cells or tissue samples. Analyze the fluorescent signal using fluorescence microscopy, a fluorescent plate reader, or by flow cytometry. Covalently Label Active Caspase 9 Whole, Living Cell Assay with Cell-permeant Regents Counter-stain with other Fluorophores Distinguish Apoptosis from Necrosis FLICA (Fluorescent Labeled Inhibitor of Caspases) probes are comprised of an caspase-inhibitor affinity peptide sequence, a fluoromethyl ketone(FMK) moiety that facilitates an irreversible caspase binding event, and a fluorescent tag (either carboxyfluorescein or sulforhodamine B) reporter. For a caspase 9 inhibitor, the enzyme recognition sequence is leucine-glutamic acid-histidine-aspartic acid (LEHD). The FLICA probe interacts with the enzymatic reactive center of an activated caspase via the recognition sequence, forming a covalent thioether adduct with the enzyme through the FMK moiety and allowing for easy detection after a wash step. The remaining green fluorescent signal is a direct measure of caspase-9 activity at the time the probe was added. Detection of nuclear morphology is also possible with the additional kit components Hoechst 33342 and Propidium Iodide. Background: Detect caspase-9 activity with FAM-FLICA Caspase 9 Assay Kit, Green Fluorescence. 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. 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 involved in what is referred to as the extrinsic apoptosis pathway that originates upon activation of cell surface death receptors. These monomers bind to death receptor proteins through their death effector domain (DED) structure. Caspase 9 is involved in what is known as the intrinsic apoptosis 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 Inhibitors of Caspases The FLICA methodology of caspase detection is available in kit form for assessing individual or poly-caspase activity in cultured cells and tissues. The non-toxic, cell-permeant FLICA reagent enters each cell, where it will irreversibly bind to activated caspases with a preference for its target peptide sequence. For example, active caspase 9 has a high affinity for the peptide sequence L-E-H-D, hence the usage of FAM-LEHD-FMK for caspase 9 detection. Because the FLICA reagent FAM-LEHD-FMK becomes covalently coupled to the active caspase 9 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 green fluorescent signal is a direct measure of the amount of caspase 9 activity present in the cell at the time the reagent was added. Cells that contain the bound FLICA can be analyzed by 96-well-plate based fluorometry, fluorescence microscopy, or flow cytometry. The carboxyfluorescein (FAM) FLICA reagent has an optimal excitation range from 490 - 495 nm, and emission range from 515 - 525 nm. Cells labeled with the FLICA FAM-LEHD-FMK reagent may be read immediately or preserved for 24 hours using the fixative. Unfixed samples may be subsequently analyzed with propidium iodide or Hoechst stain to detect changes in necrosis or nuclear morphology respectively. Other FLICA Caspase Detection Kits, containing the preferred caspase recognition amino acid sequences for poly caspases or caspase 1, 3, 6, 8, 9, 10, and 13, are also available with green or red fluorescence.
Product Categories/Family for FAM-FLICA assay kit	Apoptosis Assays/Apoptosis Detection

References

Slee, E. A., C. Adrain, and S. J. Martin. (1999) Serial Killers: ordering caspase activation events in apoptosis. *Cell Death Differ.* 6:1067-1074. Earnshaw, W.C., Martins, L.M., and Kaufmann, S.H. (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Ann. Rev. Biochem.* 68:383-424. Hengartner, M.O. (2000) The biochemistry of apoptosis. *Nature* 407:770-816. Degterev, A., Boyce, M., and Yuan, J. (2003) A decade of caspases. *Oncogene* 22:8543-8567. Nicholson, D.W. (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ.* 6:1028-1042. Thornberry, N.A., and Lazebnik, Y. (1998) Caspases: enemies within. *Science* 281:1312-1316. Cryns, V., and Yuan, J. (1998) Proteases to die for. *Genes Dev.* 12:1551 - 1570. Talanian, R.V., Quinlan, C., Trautz, S., Hackett, M.C., Mankovich, J.A., Banach, D., Ghayur, T., Brady, K.D., and Wong, W.W. (1997) Substrate specificities of caspase family proteases. *J. Biol. Chem.* 272:9677 - 9682. Garcia-Calvo, M., Peterson, E.P., Leiting, B., Ruel, R., Nicholson, D.W., and Thornberry, N.A. (1998) Inhibition of human caspases by peptide-based macromolecular inhibitors. *J. Biol. Chem.* 273:32608 - 32613. Rauber, P., Angliker, H., Walker, B., and Shaw, E. (1986) The synthesis of peptidylfluoromethanes and their properties as inhibitors of serine proteases and cysteine proteinases. *Biochem. J.* 239:633-640. Ekert, P.G., Silke, J., and Vaux, D.L. (1999) Caspase inhibitors. *Cell Death Differ.* 6:1081-1086. Bedner, E., Smolewski, P., Amstas, P., and Darzynkiewicz, Z. (2000) Activation of caspases measured in situ by binding of fluorochrome-labeled inhibitors of caspases (FLICA): correlation with DNA fragmentation. *Exp. Cell Res.* 259:308-313. Amstad, P.A., Yu, G., Johnson, G.L., Lee, B.W., Dhawan, S., and Phelps, D.J. (2001) Detection of caspase activation in situ by fluorochrome-labeled caspase inhibitors. *BioTechniques* 31:608-610. Smolewski, P., Bedner, E., Du, L., Hsieh, T.C., Wu, J.M., Phelps, D.J., and Darzynkiewicz, Z. (2001) Detection of caspase activation by fluorochrome-labeled inhibitors: multiparameter analysis by laser scanning cytometry. *Cytometry* 44:73-82.

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