

FAM-FLICA Caspase 3 & 7 Assay Kit | FAM-FLICA assay kit

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CATALOG #
AAA52729

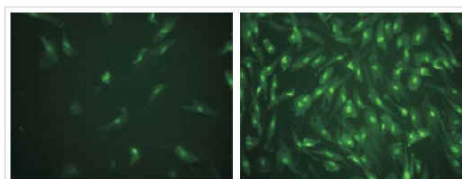
PRODUCT SYNONYMS

FAM-FLICA Caspase 3 & 7; FAM-FLICA Caspase 3 & 7 Assay Kit; FAM-FLICA assay kit

PRODUCT SPECIFICATIONS

Assay Type	Sandwich
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 3, Caspase 7
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

Normal (left) and keratoconus (right) corneal fibroblasts were treated with 200uM H₂O₂ for 1hr, washed, and allowed to recover for 1-3hrs. The culture media was removed and replaced with 1x FAM-FLICA Caspases 3&7 solution (in culture media) at 300ul/well for 1hr. The cell layer was washed 3times with 1x wash buffer; 300ul wash buffer was added to keep the cells from drying. Keratoconus corneal fibroblasts treated with H₂O₂ (right) show a significant increase in caspases 3&7 activity compared to normal cells (left). Non-apoptotic cells are dark in background. Data courtesy of Dr. Cristina Kenney, M.D., Ph.D. Dept. of Ophthalmology, UC Irvine.

ADDITIONAL INFORMATION

Related Product Information for FAM-FLICA assay kit	Background: FLICA is a powerful method to assess cell death by detecting apoptosis in vitro. probes are non-cytotoxic Fluorescent Labeled Inhibitors of Caspases that covalently bind to active caspase enzymes^{1,2}. FLICA measures the intracellular process of apoptosis instead of a sideeffect, such as the turn-over of phosphatidyl serine, and eliminates the incidence of false positives that often plagues methods like Annexin V and TUNEL staining. FLICA can also be used to measure pyroptosis, a highly inflammatory form of programmed cell death. To use FLICA, add it directly to the cell culture media, incubate, and wash. FLICA is cell-permeant and will efficiently diffuse in and out of all cells. If there is an active caspase enzyme inside the cell, it will covalently bind with FLICA and retain the green fluorescent signal within the cell. Unbound FLICA will diffuse out of the cell during the wash steps. Apoptotic and pyroptotic cells will retain a higher concentration of FLICA and fluoresce brighter than healthy cells. There is no interference from pro-caspases or inactive forms of the enzymes. If the treatment is causing cell death via apoptosis and/or pyroptosis, the cells will contain an elevated level of caspase activity relative to negative control cells and fluoresce with FLICA. Apoptosis is an evolutionarily conserved process of programmed cell suicide. It is centered 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 cell³. Caspases have been identified in organisms ranging from <i>C. elegans</i> to humans. Mammalian caspases play distinct roles in both apoptosis and inflammation. In apoptosis, effector caspases (-3, -6, and -7) are responsible for proteolytic cleavages that lead to cell disassembly. Initiator caspases (-8, -9, and -10) regulate apoptosis upstream. Caspase-1 is associated with pyroptosis and inflammasome activity and takes on the role of a key housekeeping enzyme in its conversion of pro-IL-1β protein into the active IL-1β cytokine. (Use FLICA kits to detect caspase-1.) Please note that macrophages and monocytes have been shown to rapidly secrete caspase-1 upon activation^{4,5}. Like the majority of other proteases, caspases are synthesized as pro-form precursors that undergo proteolytic maturation, either autocatalytically or in a cascade by enzymes with similar specificity⁶. Active caspase enzymes consist of two large (~20 kD) and two small (~10 kD) subunits that non-covalently associate to form a two heterodimer, tetrameric active caspase⁷⁻⁹. Activated caspase enzymes cleave proteins by recognizing a 3 or 4 amino acid sequence that must include an aspartic acid (D) residue in the P1 position. This C-terminal residue is the target for the cleavage reaction at the carbonyl end¹⁰. Each FLICA probe contains a 3 or 4 amino acid sequence that is targeted by different activated caspases. This target sequence is sandwiched between a green fluorescent label, carboxyfluorescein (FAM), and a fluoromethyl ketone (FMK). A caspase enzyme cannot cleave the FLICA inhibitor probe; instead, it forms an irreversible covalent bond with the FMK on the target sequence and becomes inhibited from further enzymatic activity. # poly caspase FLICA probe, FAM-VAD-FMK, can be used as a general reagent to detect apoptosis as it is recognized by many types of activated caspases. To more specifically target a particular caspase enzyme, use one of ?) ? specialized FLICA reagents. ?) ? #. kits for the detection of: caspase-1 (YVAD or WEHD) (also recognizes caspases 4 and 5), -2 (VDVAD), -3/7 (DEVD), -6 (VEID), -8 (LETD), -9 (LEHD), and -10 (AEVD). FLICA kits are also available with a red or far red fluorescent label. Caspases, like most other crucial cell survival enzymes, are somewhat permissive in the target amino acid sequence they will recognize and cleave. Although FLICA reagents contain the different amino acid target sequences preferred by each caspase, they can also recognize other active caspases when they are present. ?) ? encourages validation of caspase activity by an orthogonal technique. FLICA can be used to label suspension or adherent cells and thin tissue sections. After labeling with FAM-FLICA, cells can be fixed or frozen. For tissues that will be paraffin-embedded after labeling, use red sulforhodamine SR-FLICA probes; do not use the green FAM-FLICA probes as the FAM dye will be quenched during the paraffin embedding process. Cells labeled with FAM-FLICA can be counter-stained with reagents such as the red live/dead stains Propidium Iodide (included in FAMFLICA kits) and 7-AAD to distinguish apoptosis from necrosis. Nuclear morphology can be concurrently observed using Hoechst 33342, a blue DNA binding dye (included in FLICA kits). Cells can be viewed directly through a fluorescence microscope (Figures 1-3,7, and 9-10), or the fluorescence intensity can be quantified using a flow cytometer (Figures 4-6) or fluorescence plate reader (Figure 8). FAM-FLICA optimally excites at 488-492 nm and has a peak emission at 515-535 nm.
Product Categories/Family for FAM-FLICA assay kit	Apoptosis Assays/Apoptosis Detection

References

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For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/assay-kits/fam-flica-caspase-3-7/52729>