

FLICA 660 Poly Caspase Assay Kit | FLICA assay kit

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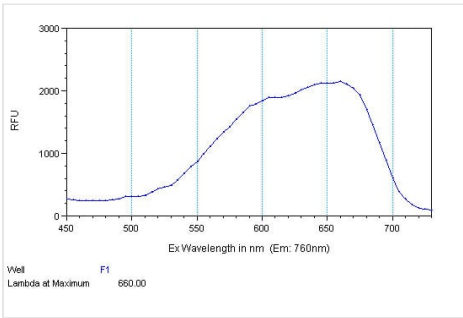
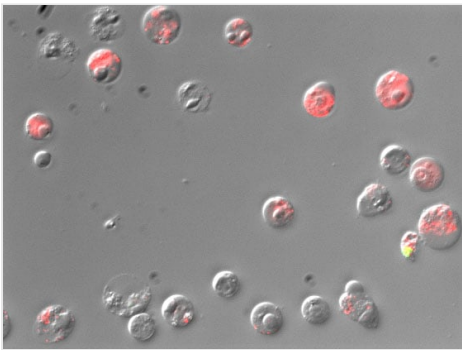
CATALOG #
AAA52719

PRODUCT SYNONYMS

FLICA 660 Poly Caspase; FLICA 660 Poly Caspase Assay Kit; FLICA assay kit

PRODUCT SPECIFICATIONS

Reagents	660-VAD-FMK
Sample Protocol	Sample Protocol: FLICA (Fluorescent-Labeled Inhibitor of Caspases) assays offer a simple yet accurate method to measure individual or pan-caspase activity in whole cells. Two sample protocols are outlined below. Detailed instructions are supplied with the kit.
Suspension Cells	Suspension Cells Culture your cells to a concentration of $2-5 \times 10^5$ cells/mL. Prepare experimental and control populations. Reconstitute the reagent with 50uL DMSO to form the stock concentrate (may be frozen for future use). Dilute the stock concentrate with 200uL PBS to form the working solution. Add working solution directly to samples and controls at a ratio of 1:30 - 1:60. E.g., add 5-10 uL of working solution to a 300 uL aliquot of suspension cells. Flow cytometry requires less reagent; microscopy applications require more reagent per sample. Incubate 15 - 45 minutes. Wash and spin cells two or three times. If desired, label cells with Hoechst stain, DAPI, or other compatible fluorescent markers. If desired, fix cells with fixative included in kits. Analyze data using a fluorescence microscope or flow cytometer.
Frozen Tissue	Tissue Sections reprepare experimental and control populations. Prepare thin frozen tissue sections appropriate to the experiment. Allow sections to thaw before staining with FLICA reagent. Reconstitute each vial of FLICA with 50 uL DMSO to form the stock concentrate. Dilute FLICA stock concentrate 1:50 in PBS to form the tissue section staining solution (TSSS). Use TSSS within 15 minutes of preparation. Add enough TSSS to cover the surface of the tissues and incubate 30 - 60 minutes protected from light. Wash with TBSt, PBSt, or 1X Apoptosis Wash Buffer (twice for 5 min). Set slides in slide incubation dish containing 1X Apoptosis Wash Buffer. Stain nuclei with DAPI or Hoechst, and apply coverslip. Image with appropriate fluorescence microscopy filters. Store samples at 2-8 degree C for short-term storage; staining will last at -20 degree C for long periods.
Target	Poly Caspases
Excitation / Emission	660 nm / 680-690 nm
Method of Analysis	Flow Cytometer, Fluorescence Microscope
Types of Samples	Cell Culture, Tissue
Preparation and Storage	Store at 2-8 degree C



Application Data
FLUORESCENCE PROPERTIES OF FLICA 660POLY CASPASE REAGENT:Excitation max:660 nmEmission max: 690nm

Application Data
INTRACELLULAR APOPTOSIS DETECTION VIA ACTIVE CASPASE ASSAY To demonstrate the fluorescence properties and apoptosis detection capability of the new far-red fluorescent FLICA 660 Poly Caspase Assay, Jurkat cells were pretreated with 1 μ M staurosporine for 4 hours and labeled with FLICA 660-VAD-FMK poly caspase inhibitor reagent. Using a Nikon E800 Hyperspectral Microscope equipped with a Photometrics HQ2 camera, far-red fluorescence emission from apoptotic Jurkat cells was acquired with a Cy5 HYQ bandpass filter combination set. FLICA 660-VAD-FMK reagent optimally excites at 660 nm and has an emission max at 690 nm, making it suitable for use with red lasers on common benchtop flow cytometers and ideal for dual staining purposes with green fluorescent probes.

ADDITIONAL INFORMATION

Related Product Information for FLICA assay kit

Background: FLICA is a powerful method to assess caspase activity. FLICA probes are cell permeant, noncytotoxic Fluorescent Labeled Inhibitors of CASpases that covalently bind with active caspase enzymes^{1, 2}. AAA Biotech, Inc. has developed a far-red excitation and emission spectra FLICA 660 probe for the detection of cells bearing active caspases. 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. 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 specificity³. 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 caspase^{4, 5, 6}. Once activated, caspases cleave protein substrates leading to the eventual disassembly of the cell. Caspases have been identified in organisms ranging from *C. elegans* to humans. Mammalian caspases play distinct roles in both apoptosis and inflammation. FLICA 660 poly caspase inhibitor probe contains the preferred binding sequence for all caspases, Val-Ala-Asp (VAD). This preferred poly caspase tripeptide binding sequence is labeled at the amino terminus end with a far-red fluorescent 660 dye and linked at the carboxyl end to a fluoromethyl ketone (FMK) reactive entity. The resulting cell permeant, fluorescent molecule, 660-VAD-FMK, optimally excites at 660 nm and emits between 685-690 nm. A conventional red HeNe laser with a 633 nm excitation provides excellent excitation efficiency, enabling cells labeled with FLICA 660 to be analyzed with most flow cytometers and fluorescence microscopes equipped with electronic grey scale image capabilities. To use FLICA, add it directly to the cell 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 660-VAD-FMK and retain the far-red fluorescent signal within the cell. Unbound FLICA will diffuse out of the cell during the wash steps. Apoptotic cells will retain a higher concentration of FLICA and fluoresce brighter than non-apoptotic cells. There is no interference from pro-caspases or inactive forms of the enzyme. If the treatment is causing cell death via apoptosis, apoptotic cells will have an elevated level of caspase activity relative to non-apoptotic or negative control cells and fluoresce with FLICA. After labeling with FLICA, cells can be counter-stained with other reagents and fixed or frozen.

Product Categories/Family for FLICA assay kit

FLICA 660 Poly Caspase Assay Kit; far-red fluorescence

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.