

FAM-Leu-CMK Green FLISP Assay Kit | FAM-Leu-CMK assay kit

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CATALOG #
AAA52725

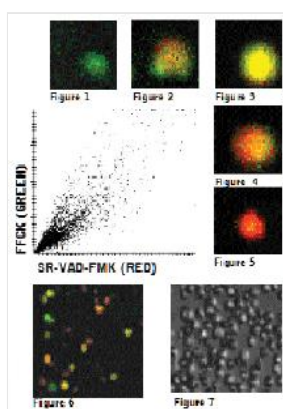
PRODUCT SYNONYMS

FAM-Leu-CMK Green FLISP; FAM-Leu-CMK Green FLISP Assay Kit; FAM-Leu-CMK assay kit

PRODUCT SPECIFICATIONS

Reagents	FLCK
Sample Protocol	Culture cells up to 1×10^6 cells/mL. Follow your experimental protocol where chymotrypsin-like enzyme activity will be investigated; create positive and negative controls for comparing chymotrypsin activity. Reconstitute the vial of FLISP reagent with the appropriate volume of DMSO to form the stock concentrate (can be frozen for future use). Dilute the stock concentrate with PBS to form the 50X sample spiking solution. Add 6-10 uL of the FLISP spiking solution directly to a 300-500 uL aliquot of your cell culture for labeling. Incubate 30 minutes -1 hour at 37 degree C in the dark. Wash cells 3X with fresh media or 1x working dilution of FLISP wash buffer. If desired, label cells with Hoechst stain. If desired, label cells with Propidium Iodide. If desired, fix cells for future analysis. Analyze data using a fluorescence microscope, plate reader, or flow cytometer.
Target	Chymotrypsin-like enzymes
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	FLISP at -20 degree C Other Kit Components at 2 - 8 degree C

IMAGES



Application Data

HL-60 cells were labeled with FAM-FLISP and ICT's SR-VAD-FMK FLICA caspase reagent (kit #917) after incubation with camptothecin for 3 hours. Cells with active serine proteases stain green (FAM-FLISP, y-axis, Fig. 1) and cells with active caspases stain red (SR-VAD-FMK, x-axis, Fig. 5). Co-localization of serine protease activity versus caspase activity is evident in dually stained cells (Figs. 2, 3, 4, & 6). The DIC image (Fig. 7) reveals many negative cells (Dr. Zbigniew Darzynkiewicz, Brander Cancer Center).

ADDITIONAL INFORMATION

Related Product Information for FAM-Leu-CMK assay kit	Description: Detect changes in intracellular chymotrypsin-like serine protease enzyme activity in whole living cells with the FLISP, Fluorescent-Labeled Inhibitors of Serine Proteases, product line of assay kits. This assay's FLISP reagent, FLCK, utilizes a leucine (L) chymotrypsin-targeting amino acid residue linked on the amino terminus with a carboxyfluorescein (FAM) fluorescent reporter tag. The carboxyl end of the L amino acid residue contains a chloromethyl ketone (CMK) reactive group that targets the catalytic site of serine proteases. After quickly penetrating the lipid bilayer membranes of the target cell population, the chymotrypsin-targeting FLISP probe FLCK will interact with the active catalytic sites of chymotrypsin-like proteases, quickly forming covalent bonds with either the reactive site histidine (N-H). Unbound FLISP reagent is easily removed during the wash step, leaving cells with greater quantities of active chymotrypsin-like enzyme activity showing a greater fluorescence potential than cells that did not undergo an upregulation of serine protease activity. The green FAM reporter dye is excited at 488 nm and emits in the green wavelength range of 525 nm. FLISP probes are cell permeant and non-cytotoxic at the concentrations suggested in the assay protocol. The FLISP FAM-Leu-CMK (FLCK) chymotrypsin enzyme detection assay kits can be used in conjunction with existing apoptosis detection protocols. They have been used successfully in tandem with the red SR FLICA caspase detection assay kits to show a parallel upregulation of chymotrypsin-like enzyme activity and caspase activation. Each kit includes the FLISP FLCK reagent, 10x wash buffer for removing excess FLISP probe following the FLISP incubation step, 10X fixative to stabilize cells if next day analysis is preferred, Propidium Iodide vital stain for necrotic cell detection, and Hoechst 33342 dye to visualize nuclear morphology. FLISP FLCK-stained cells may be analyzed using flow cytometry, fluorescence microscopy, and fluorescence plate reader evaluation methodology. Background: Chymotrypsin-like enzymes cleave their substrate proteins at the carboxy terminal end of amino acids containing hydrophobic aliphatic or aromatic R-group side chains such as those found on leucine and phenylalanine amino acid structures [1]. The FLCK FLISP probe, consisting of FAM-L-CMK, contains a leucine amino acid rather than a phenylalanine in the P1 position of the FLISP probe. Because leucine also contains a hydrophobic R-group side chain structure, it could also represent a type of fluorescent labeled analog of the early chymotrypsin-like enzyme inhibitor N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) [2]. Early studies using topoisomerase inhibitors in HL-60 cells indicated that TPCK, N-tosyl- L-lysine chloromethyl ketone (TLCK) and other serine protease inhibitors were able to prevent internucleosomal DNA degradation associated with apoptosis [3]. Camptothecin induction experiments using HL-60 cells indicated a high linear correlation ($r = 0.98$) between apoptosis induction as measured by FAM-VAD-FMK binding to cells and FLISP probe binding frequency [4]. Dual staining experiments were also run using FAM-F-CMK or FAM-L-CMK (chymotrypsin detection probes) and the sulforhodamine B (SR)-VAD-FMK FLICA caspase detection probe [4-5]. This comparison showed a correlation between green fluorescence intensity staining from FAM-F-CMK ($r = 0.72$) or FAM-L-CMK ($r = 0.82$) stained cells and red stained SR-VAD-FMK stained cells. This group also showed that the chymotrypsin-like enzyme inhibitor, TPCK, had a strong suppressive effect on caspase activation and apoptosis induction. In contrast, the trypsin-like enzyme inhibitor, TLCK, had very little effect on caspase activation events as measured by the binding of the red FLICA probe SR-VAD-FMK in camptothecin-induced cell populations [4].
Product Categories/Family for FAM-Leu-CMK assay kit	Apoptosis Assays/Serine Protease Detection
References	Czapinska, H. and J. Otlewski. 1999. Structural and energetic determinants of the S1-site specificity in serine proteases. <i>Eur. J. Biochem.</i> 260: 571-595. Grabarek, J., M. Dragan, B.W. Lee, G.L. Johnson, and Z. Darzynkiewicz. 2002. Activation of chymotrypsin-like serine proteases during apoptosis detected by affinity-labeling of the enzymatic center with fluoresceinated inhibitor. <i>Int. J. Oncol.</i> 20: 225-233. Zhu, H., D. Dinsdale, E.S. Alnemri, and G.M. Cohen. 1997. Apoptosis in human monocytic THP-1 cells involves several distinct targets of N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK). <i>Cell Death Diff.</i> 4: 590-599. Grabarek, J., L. Du, G.L. Johnson, B.W. Lee, D. J. Phelps, and Z. Darzynkiewicz. 2002. Sequential activation of caspases and serine proteases (Serpases). <i>Cell Cycle</i> 1: 124-131. Grabarek, J., M. Dragan, B.W. Lee, G.L. Johnson, and Z. Darzynkiewicz. 2002. Activation of chymotrypsin-like serine protease(s) during apoptosis detected by affinity-labeling of the enzymatic center with fluoresceinated inhibitor. <i>Int. J. Oncol.</i> 20: 225-233.

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.