

FAM-Spacer-Phe-CMK Green FLISP Assay Kit | FAM-Spacer-Phe-CMK assay kit

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CATALOG #
AAA52709

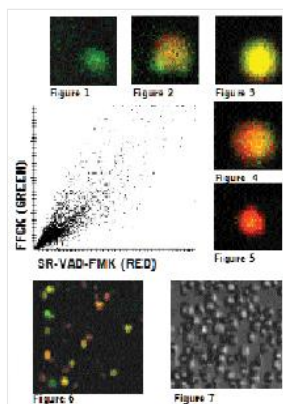
PRODUCT SYNONYMS

FAM-Spacer-Phe-CMK Green FLISP; FAM-Spacer-Phe-CMK Green FLISP Assay Kit; FAM-Spacer-Phe-CMK assay kit

PRODUCT SPECIFICATIONS

Reagents	FSFCK
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 μ L of the FLISP spiking solution directly to a 300-500 μ L 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 FAM-spacer-F-CMK (one 25-test vial) Hoechst Stain (1 mL) Propidium Iodide (1 mL) 10x FLISP Wash Buffer (15 mL) Fixative (6mL)
Preparation and Storage	CFSE at -20 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-Spacer-Phe-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 utilizes a reagent (FSFCK) containing a phenylalanine (F) chymotrypsin-targeting amino acid residue linked on the amino terminus with a carboxyfluorescein (FAM) fluorescent reporter tag. The carboxyl end of the F amino acid residue contains a reactive chloromethyl ketone (CMK) 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 FSFCK will interact with the active catalytic sites of chymotrypsin-like proteases, quickly forming covalent bonds with the reactive site histidine (N-H). Unbound FLISP reagent is easily removed during the wash step, resulting in 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. Green FAM-FLISP probes like FSFCK are excited at 488 nm and emit in the green wavelength range of 525 nm. Red fluorescence FLISP probes are also available. FLISP probes are cell permeant and non-cytotoxic at the concentrations suggested in the assay protocol. The FAM-spacer-F-CMK chymotrypsin enzyme detection assay kits can be used in conjunction with existing apoptosis detection protocols. Each FLISP FSFCK kit includes either 25 tests or 100 tests of FAM-spacer-F-CMK reagent, 10X wash buffer, 10X fixative to stabilize cells if needed for next day analysis, PI vital stain for necrotic cell detection, and Hoechst 33342 dye to stain apoptotic nuclei. FSFCK-stained cells can 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]. This FLISP probe, consisting of FAM-spacer-Phe-CMK, is identical to the FAM-Phe-CMK probe except FAM-spacer-Phe-CMK includes an additional six carbon spacer group to minimize possible steric hindrance issues. Both the FAM-spacer-Phe-CMK and FAM-Phe-CMK probes are fluorescent-labeled analogs of the early chymotrypsin-like enzyme inhibitor N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) [2]. When the two versions of this FAM-Phe-CMK probe (either with or without the six carbon spacer option) were compared in staurosporine induction experiments in Jurkat cells, there were no detectable differences in the chymotrypsin-like enzyme labeling pattern between the two forms of the phenylalanine containing FLISP probe. 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]. Dual staining experiments have used our initial chymotrypsin detection probe, FAM-Phe-CMK, and caspase detection probe SR-VAD-FMK in the presence or absence of z-VAD-FMK, TPCK, or TLCK caspase or serine protease inhibitors with published success [2,4]. This work demonstrated a significant inhibitory effect of the caspase inhibitor, z-VAD-FMK, on chymotrypsin-associated FAM-Phe-CMK binding in 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 [5]. The FAM-Phe-CMK chymotrypsin-like protein affinity label was also utilized to analyze up-regulated serine protease activity resulting from staurosporine induction in HL-60 and Jurkat cells [6-7]. In staurosporine-induced Jurkat cells, the FAM-Phe-CMK specifically labeled an up-regulated, constitutively expressed, 60 kDa protein as well as a 50 kDa protein that was only detected in staurosporine treated Jurkat cells [6]. In a related study using staurosporine induced HL-60 cells, a 16 kDa protein was detected by the FAM-F-CMK FLISP type probe [7].
Product Categories/Family for FAM-Spacer-Phe-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. Eur. J. Biochem. 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. Int. J. Oncol. 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). Cell Death Diff. 4: 590-599. 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. Int. J. Oncol. 20: 225-233. 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). Cell Cycle 1: 124-131. O'Connell, A.R., B.W. Lee, and C. S. Cox. 2006. Caspase-dependent activation of chymotrypsin-like proteases mediates nuclear events during Jurkat T cell apoptosis. Biochem. Biophys. Res. Comm. 345: 608-616. O'Connell, AR., C. Holohan, A. Torriglia, B.W. Lee, and C.S. Cox. 2006. Characterization of a serine protease-mediated cell death program activated in human leukemia cells. Exp. Cell Res. 312: 27-39.

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-spacer-phe-cmk-green-flisp/52709>