

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

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CATALOG #
AAA52715

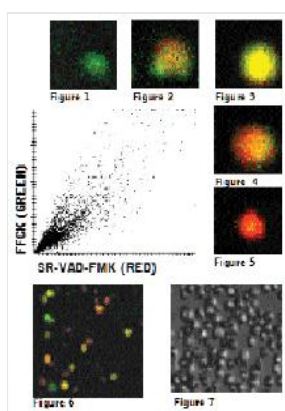
PRODUCT SYNONYMS

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

PRODUCT SPECIFICATIONS

Reagents	FFCK
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 FFCK and ICT's SR-VAD-FMKFLICA caspase reagent (kit #917) after incubation with camptothecin for 3 hours. Cells with active serine proteases stain green (FFCK, 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-Phe-CMK assay kit	Description: Detect changes in intracellular chymotrypsin-like enzyme activity in whole living cells with the FLISP, Fluorescent-Labeled Inhibitors of Serine Proteases, product line of assay kits. These kits utilize either a leucine (L) or phenylalanine (F) chymotrypsin-targeting amino acid residue linked on the amino terminus with either a carboxyfluorescein (FAM) or sulforhodamine 101 (SR) fluorescent reporter tag. The carboxyl end of the F or L amino acid residue contains a reactive group that targets the catalytic site of serine proteases, consisting of either a chloromethyl ketone (CMK) or (aminoalkyl) phosphonate diphenyl ester (DAP) reactive group. After quickly penetrating the lipid bilayer membranes of the target cell population, the chymotrypsin-targeting FLISP probes will interact with the active catalytic sites of chymotrypsin-like proteases, quickly forming covalent bonds with either the reactive site histidine (N-H) (with the CMK-type FLISP probes) or the reactive serine OH group (when DAP-containing FLISP probes are utilized). In either case, unbound FLISP reagent is easily removed during the wash step, leaving cells with greater quantities of active chymotrypsin-like enzyme activity with a greater fluorescence potential than cells that did not undergo an upregulation of serine protease activity. FLISP probes bearing FAM reporter dyes are excited at 488 nm and emit in the green wavelength range of 525 nm. The red fluorescence FLISP probes that utilize sulforhodamine 101 as the reporter dye are excited at 590 nm and emit at 620 nm. FLISP probes are cell permeant and non-cytotoxic at the concentrations suggested in the assay protocol. The FLISP FAM-Phe-CMK (FFCK) chymotrypsin enzyme detection assay kits can be used in conjunction with existing apoptosis detection protocols. They have been used successfully in tandem with the SR (red) FLICA caspase detection assay kits to show a parallel upregulation of chymotrypsin-like enzyme activity and caspase activation. Each kit includes the FLISP FFCK 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 FFCK-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 FFCK FLISP probe, consisting of FAM-F-CMK, is simply a 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]. Dual staining experiments have used this FAM-F-CMK chymotrypsin detection probe and the sulforhodamine (SR)-VAD-FMK FLICA caspase detection probe in the presence or absence of z-VAD-FMK, TPCK, or TLCK caspase or serine protease inhibitors [4-5]. This work demonstrated a significant inhibitory effect of the caspase inhibitor, z-VAD-FMK, on chymotrypsin associated FAM-F-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-F-CMK chymotrypsin-like protein affinity label was also utilized to analyze upregulated serine protease activity resulting from staurosporine induction in HL-60 and Jurkat cells [6-7]. In staurosporine-induced Jurkat cells, the FAM-F-CMK specifically labeled an upregulated, 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 probe [7].
Product Categories/Family for FAM-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-phe-cmk-green-flisp/52715>