

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

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CATALOG #
AAA52724

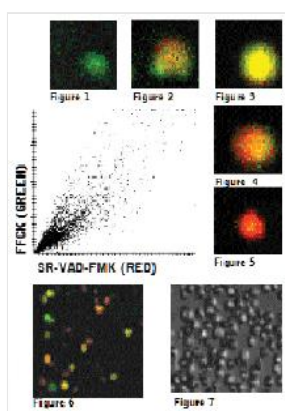
PRODUCT SYNONYMS

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

PRODUCT SPECIFICATIONS

Reagents	FLDAP
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 dual-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-DAP 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 leucine (L) chymotrypsin-targeting amino acid residue linked on the amino terminus with either a carboxyfluorescein (FAM) 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 an aminoalkyl phosphonate diphenyl ester (DAP) reactive group. After quickly penetrating the lipid bilayer membranes of the target cell population, the chymotrypsin-targeting FLISP probe will interact with the active catalytic sites of chymotrypsin-like proteases, quickly forming covalent bonds with 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 FAM-L-DAP chymotrypsin enzyme detection assay kits can be used in conjunction with your existing apoptosis detection protocols. They have been used successfully in tandem with the red SR FLICA apoptosis detection assay kits to show a parallel upregulation of chymotrypsin-like enzyme activity and caspase activation. Each kit includes either 25 tests or 100 tests of FAM-L-DAP reagent, 10X wash buffer for removing excess FLISP probe following the FLISP incubation step, 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. FAM-L-DAP 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 terminus of amino acids containing hydrophobic aliphatic or aromatic R-group side chains such as those found on leucine and phenylalanine amino acid structures [1]. Compared to our FAM-F-DAP FLISP probe, the FAM-L-DAP FLISP probe contains a leucine amino acid rather than a phenylalanine in the P1 position. Because both leucine and phenylalanine contain a hydrophobic R-group side chain structure, they could both 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]. Dual staining experiments have used our original green FLISP chymotrypsin detection probe (FAM-F-CMK) and red FLICA poly caspase detection probe in the presence or absence of z-VAD-FMK, TPCK, or TLCK caspase or serine protease inhibitors with published success [2,4]. alpha-Aminoalkyl phosphonates were developed to address the need for reagents capable of covalently labeling the reactive serine-OH amino acid groups within the catalytic sites of serine proteases [5-6]. Chloromethyl ketone-based inhibitors very efficiently perform the task of labeling serine proteases but also have the capability of reacting with other enzymes and proteins in a non-specific manner and are more subject to hydrolysis over time in aqueous/cell culture media environments [7]. FLISP probes, containing this peptidyl ?-aminoalkylphosphonate (DAP) organophosphorus inhibitor structure, seek to satisfy the need for a reactive yet specific mechanism for labeling chymotrypsin-like serine proteases in whole living cells.
Product Categories/Family for FAM-Leu-DAP 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., 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. Oleksyszyn, J., and J. Powers. 1991. Irreversible inhibition of serine proteases by peptide derivatives of (?-aminoalkyl)phosphonate diphenyl esters. Biochemistry 30: 485-493. Abuelyaman, A.S., D.S. Jackson, D. Hudig, S.L. Woodard, and J.C. Powers. 1997. Synthesis and kinetic studies of diphenyl 1-(N-peptidylamino) alkanephosphonate esters and their biotinylated derivatives as inhibitors of serine proteases and probes for lymphocyte granzymes. Arch. Biochem. Biophys. 344: 271-280. Powers, J.C., J.L. Asgian, O.D. Ekici, and K.E. James. 2002. Irreversible inhibitors of serine, cysteine, and threonine proteases. Chem. Rev. 102: 4639-4750.

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.

