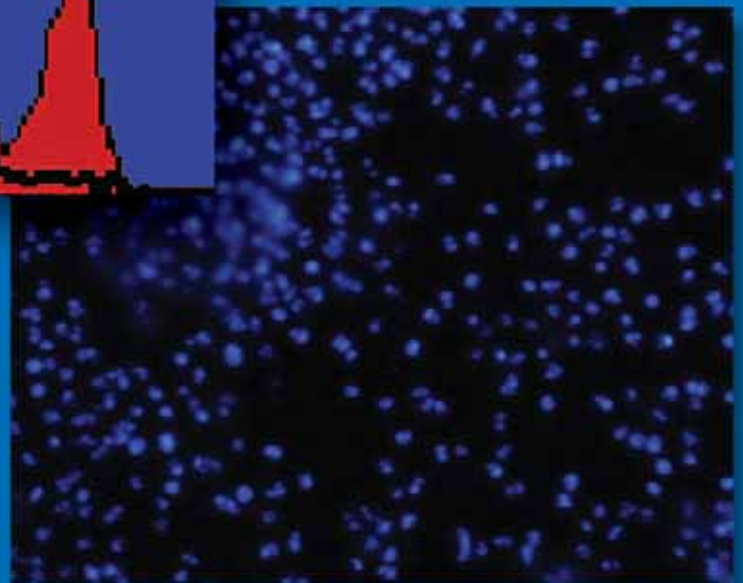
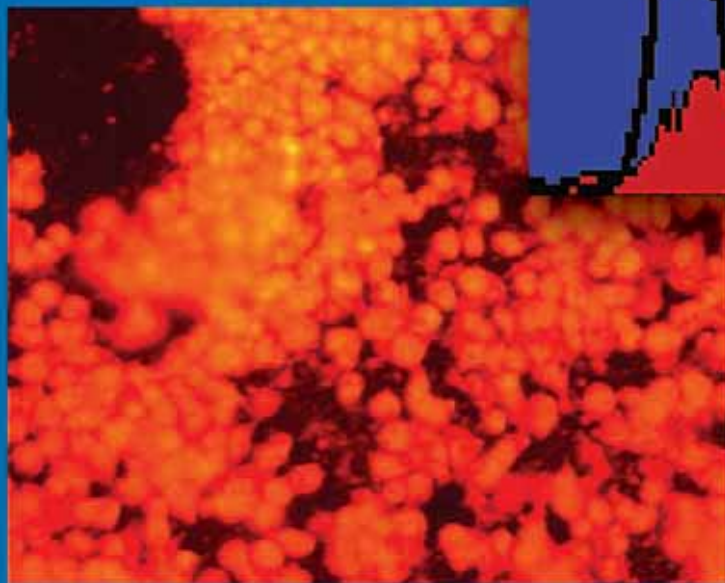
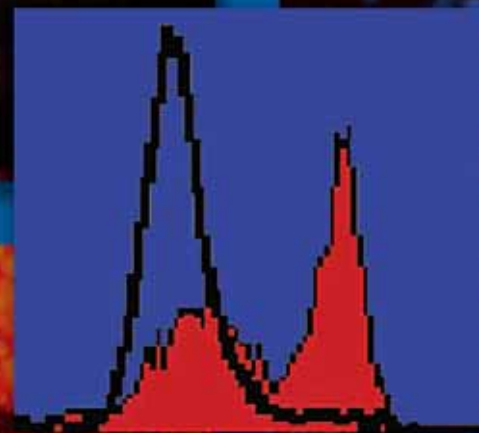
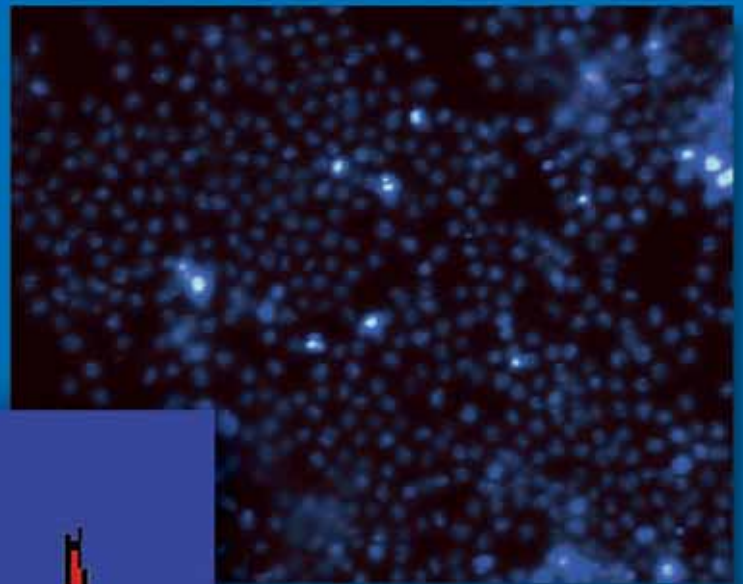
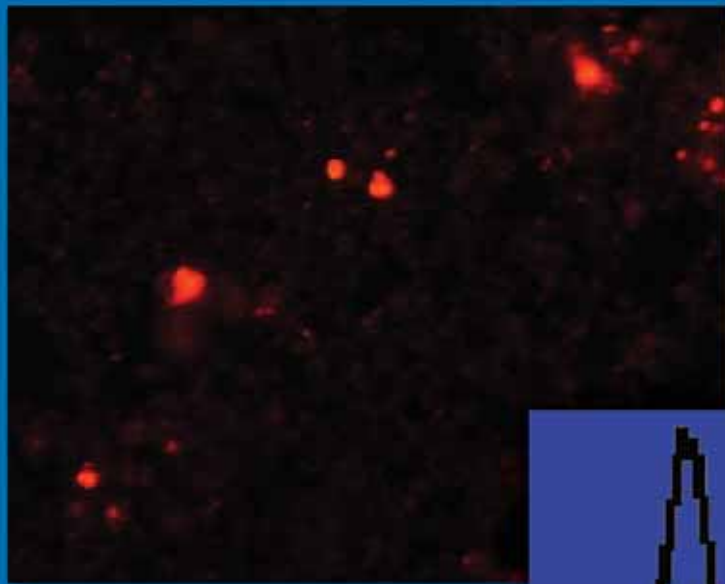


Innovative Inhibitor Products for Research

Active Serine Protease Kits

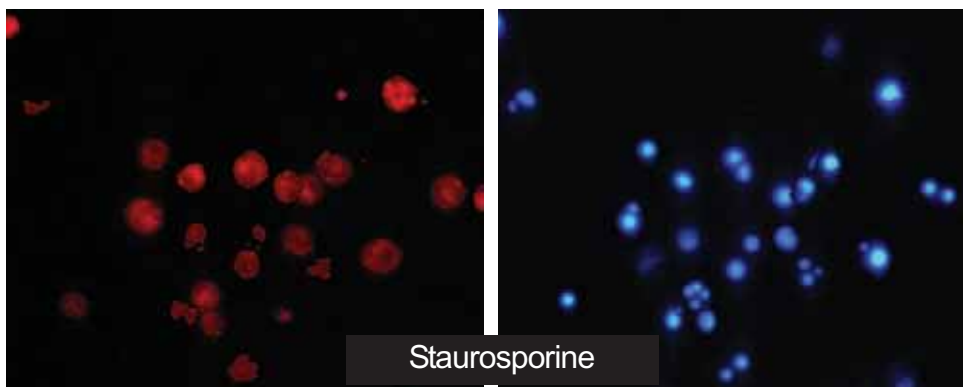
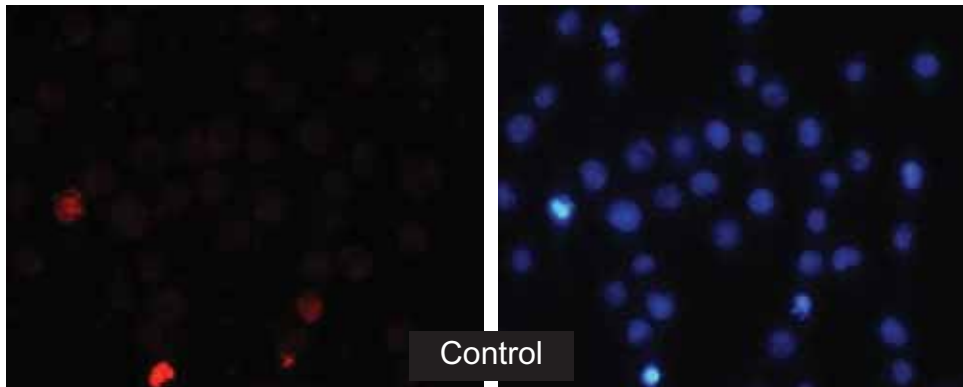


Activation of serine proteases has been described during apoptosis

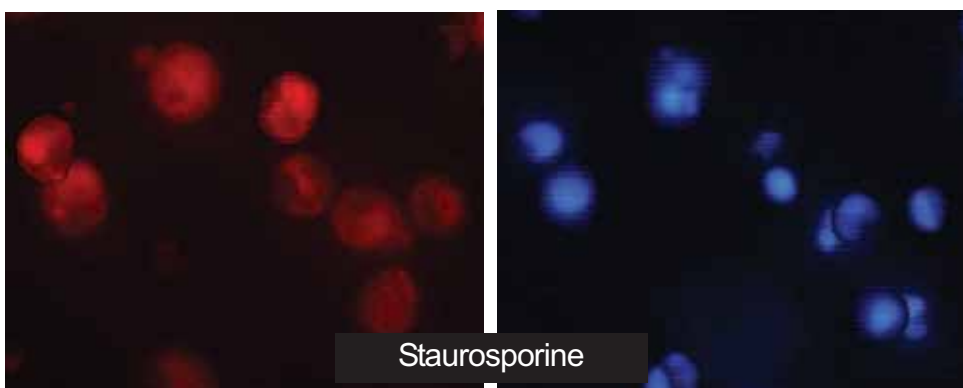
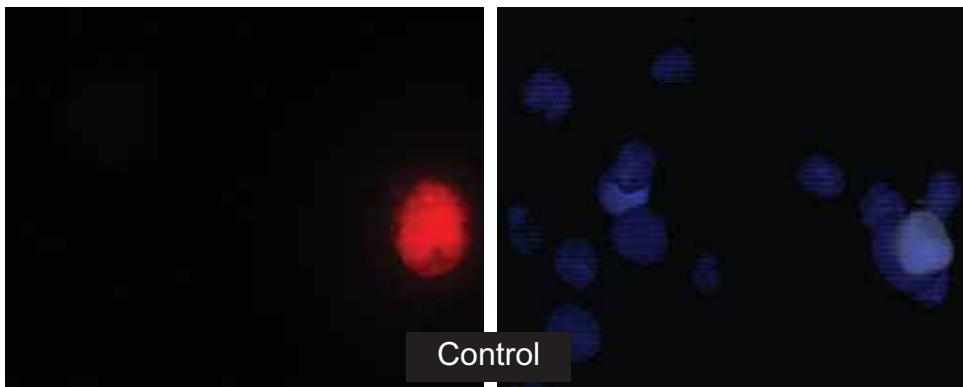
Jurkat

TR-Phe-CMK

Hoechst



TR-Phe-CMK staining in the control population corresponded to cells with condensed chromatin (bright blue). In contrast, TR-Phe-CMK stained the majority of staurosporine treated cells. (100X magnification). Activation of serine proteases has been described during apoptosis (1, 2). Cells stained in the control population may represent spontaneous apoptosis.



Higher magnification (400X) showed that the brightness and subcellular localization of TR-Phe-CMK varied between cells.

Cover Art

TR-Phe-CMK detection of active serine proteases by fluorescence microscopy (red) and flow cytometry in control and staurosporine-treated HeLa cells. (1 μ M/16 h). Hoechst DNA counterstaining (blue). Control cells: top micrographs and unshaded histogram. Staurosporine-treated cells: bottom micrographs and shaded histogram.

Introduction

Serine proteases belong to a multigene family of proteins that include the digestive enzymes trypsin, chymotrypsin and elastase. Serine proteases, like other proteases, are synthesized as larger pro-enzyme forms. During activation the pro-enzyme is cleaved resulting in the active enzyme. Serine proteases typically include a serine, a histidine, and an aspartate residue in the active site region of the enzyme, although some members may utilize glutamic or lysine groups. Serine proteases differ in their substrate specificity through variations in the amino acid composition of the S1 binding region of the enzyme. Activated proteases play key roles in regulating signal transduction pathways by proteolytically cleaving and activating downstream protein substrates, including other proteases. This results in amplification of signaling cascades. Protease cleavage also degrades protein substrates, which acts as another key mechanism in regulating signaling pathways.

A growing body of evidence indicates that the chymotrypsin-like serine protease subfamily, in addition to their well-documented roles in the digestive process, plays a key role in a number of diverse physiological processes, including the maintenance of homeostasis, inflammation and immune reactions, apoptosis, and cell survival. For example, chymotrypsin-like serine proteases have been shown to be activated during apoptosis signaling (1,2). Additionally,

the chymotrypsin serine protease inhibitor 1-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) has been shown to induce apoptosis by blocking I κ B α degradation, which is important in the NF- κ B cell survival signaling pathway (3).

Our new SerPase™ Kits are research tools for measuring chymotrypsin-like protease activation. The technology utilizes fluorochrome-labeled inhibitors that bind specifically to the active sites of chymotrypsin-like proteases (2). The use of labeled inhibitors as activation markers, first described for measuring caspase activity, is an emerging, cutting-edge technology that has applications in intracellular flow cytometry, fluorescence microscopy, and 96-well fluorescence plate assays (2, 4). A key advantage of this technology for intracellular flow cytometry and fluorescence microscopy is the cell membrane permeability of these inhibitors. They are designed to be used with living cells and do not require any cellular fixation or permeabilization steps.

The nomenclature Serpase was first defined in analogy to caspases to describe serine proteases that are activated during apoptosis (2). However, compared to caspases much less information is known about apoptosis-associated serpases. The SerPase™ Kits should be useful research tools for helping to elucidate the roles of serine proteases in apoptosis, cell survival, and other signal transduction pathways.

Kit Contents & Descriptions

Serpase™ Kits contain either a carboxyfluorescein (FFCK) or Texas Red fluorochrome-labeled (TRFCK) inhibitor to measure green or red fluorescence, respectively. FFCK [FAM-Phe-CMK (carboxyfluoresceinyl-L-phenylalanyl-chloromethyl ketone)] and TRFCK [TR-Phe-CMK (Texas Red-L-phenylalanyl-chloromethyl ketone)] are fluorochrome-labeled analogs of TPCK (N-tosyl-L-phenylalanine chloromethyl ketone). TPCK is an inhibitor that covalently binds to the active centers of chymotrypsin-like enzymes. FFCK and TRFCK fluorescently label the active centers of serine proteases and have been used to

detect serine protease (serpase) activation in live cells by flow cytometry (1,2), fluorescence microscopy (1,2) and 96-well microtiter plate fluorometry. Cell lysates prepared from FFCK and TRFCK labeled cells have also been used to detect FFCK-reactive proteins by western blot using anti-FITC second step antibodies (1).

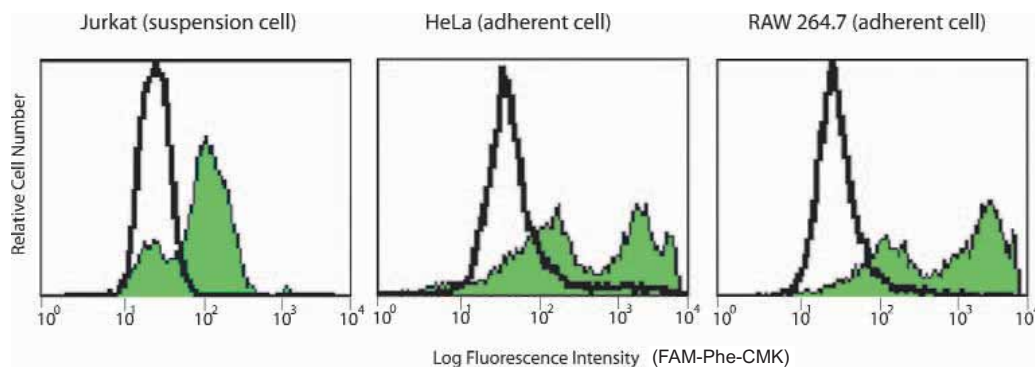
The SerPase™ Kits also contain Hoechst Stain for labeling the nuclei, Propidium Iodide for distinguishing membrane-compromised, necrotic and dead cells from healthy cells, Wash Buffer, and Fixative for an optional fixation step.

References:

1. Grabarek, J. *et al.* 2002. *Cell Cycle* 1:124-131.
2. Grabarek, J. *et al.* 2002. *International J. Oncology*. 20:225-233.
3. Bian, X. *et al.* 2002. *J. Biol Chem.* 277:42144-42150.
4. Amstad, PA *et al.* 2001. *Biotechniques* 31:608-610.

IMGENEX SerPase™ Kits

| Products | Cat. No. |
|-------------------------------|--------------|
| FAM-Phe-CMK (FFCK), 25 tests | IMI-2301-25 |
| FAM-Phe-CMK (FFCK), 100 tests | IMI-2301-100 |
| TR-Phe-CMK (TRFCK), 25 tests | IMI-2303-25 |
| TR-Phe-CMK (TRFCK), 100 tests | IMI-2303-100 |



FAM-Phe-CMK flow cytometric detection of active serine proteases in control (unshaded histograms) and staurosporine-treated (shaded histograms) human Jurkat and HeLa cells and mouse RAW cells. Staurosporine treatment (1 μ M): Jurkat, 3.5 h; HeLa and RAW, 16 h.

Cell-permeable Caspase Inhibitors

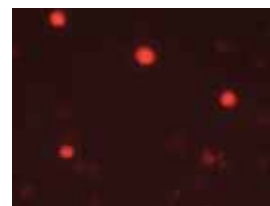
Caspase inhibitors are key research tools for studying and manipulating the process of apoptosis. Q-VD-OPH is a novel irreversible, broad spectrum caspase inhibitor specifically designed for *in vivo* research (1). Salient features include potent antiapoptotic properties (IC₅₀ values against purified caspases are in the nanomolar range), non-toxicity and enhanced cell membrane permeability compared to FMK-based inhibitors. Q-VD-OPH is also useful for *in vitro* studies (1).

References:

1. Caserta *et al.*, 2003. *Apoptosis* 8:345-352.
2. Amstad, PA *et al.* 2001. *Biotechniques* 31:608-610.

| Cat. No. | Product | Target Caspases | Applications |
|----------|---|--------------------|--|
| IMI-2309 | Q-VD-OPH 1 mg, 5 mg | Broad spectrum | <i>in vivo</i> and <i>in vitro</i> apoptosis blocking |
| IMI-2310 | Z-VAD-FMK 1 mg, 5 mg | Broad spectrum | <i>in vitro</i> apoptosis blocking |
| IMI-2311 | BOC-D-FMK 1 mg, 5 mg | Broad spectrum | <i>in vitro</i> apoptosis blocking |
| IMI-2312 | Z-DEVD-FMK 1 mg, 5mg | Caspase-3/7 | <i>in vitro</i> apoptosis blocking |
| IMI-2313 | Z-IETD-FMK 1 mg, 5 mg | Caspase-8 | <i>in vitro</i> apoptosis blocking |
| IMI-2314 | Z-LEHD-FMK 1 mg, 5 mg | Caspase-9 | <i>in vitro</i> apoptosis blocking |
| IMI-2315 | FAM Caspase Activity Kit, 25, 100 tests | Broad spectrum | flow cytometry, fluorescence microscopy, 96 well assay |
| IMI-2316 | SR Caspase Activity Kit, 25, 100 tests | Broad spectrum | fluorescence microscopy, 96 well assay |
| IMI-2317 | MitoPT™ 25, 100 tests | N/A (Mitochondria) | flow cytometry, fluorescence microscopy, 96 well assay |

Fluorochrome-labeled caspase inhibitors are novel tools for detecting and quantitating caspase activation in living cells by intracellular flow cytometry, fluorescence microscopy, and 96-well fluorescence plates. These broad spectrum caspase inhibitors (FAM and SR Caspase Activity Kits) are cell permeable and are capable of binding to the active sites of caspases. A key advantage is that neither cellular fixation nor permeabilization is needed to carry out the assays (2).



SR-VAD-FMK fluorescence microscopy detection of active caspases in Jurkat cells treated with Staurosporine (1 μM, 3 h).

Cell-permeable NF-κB Pathway Inhibitors

Genes that are regulated by nuclear factor NF-κB have been shown to suppress apoptosis, induce proliferation, and to mediate inflammation, angiogenesis, and tumor metastasis. IMGENEX has developed several inhibitors of NF-κB activation. These include inhibitory peptides, GeneSuppressor ReadyGenes, and an adenovirus-containing IκBα dominant mutant.

The inhibitory peptides are hybrid peptides containing a stretch of amino acids which confer cell permeability fol-

lowed by a short stretch of amino acid sequences from proteins involved in the NF-κB signaling.

The adenovirus IκBα mutant expresses a deleted form of IκBα in the infected cells. It competes with endogenous IκBα for binding to the IKK complex, thereby acting as a dominant negative mutant preventing phosphorylation of IκBα. This prevents translocation of p65-p50 complex into the nucleus thereby inhibiting NF-κB activation.

Human p65, includes negative control IMG-2001

Sequence DRQIKIWFQNRRMKWKKQLRRPDSRELS*

Control sequence DRQIKIWFQNRRMKWKK

Quantity 1 mg of each peptide

Human IKKγ includes negative control IMG-2000

Sequence DRQIKIWFQNRRMKWKKTALDWSWLQTE¹

Control sequence DRQIKIWFQNRRMKWKK

Quantity 1 mg of each peptide

TRAF6 binding peptide² includes negative control IMG-2002

Sequence AAVALLPAVLLALLAPRKIPTEDYTDPRPSQPST*

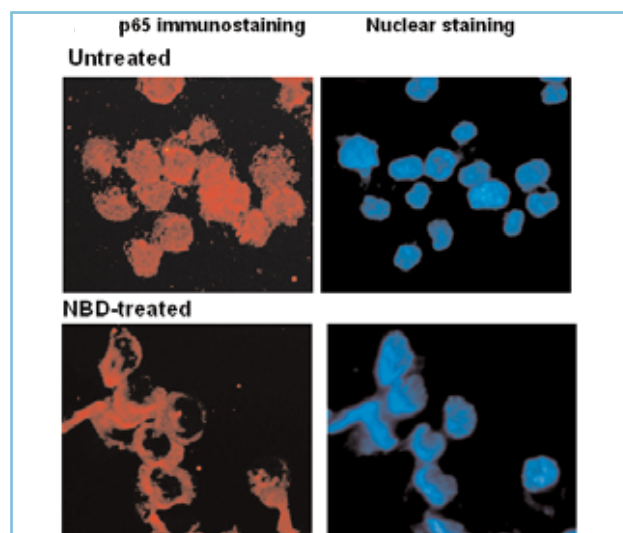
Control sequence AAVALLPAVLLALLAP

Quantity 1 mg of each peptide

*US Patents Pending

References

1. Bharti AC, *et al.* *Blood*, Vol. 101, No. 3, pp. 1053-1062 (2003)
2. Ye H, *et al.* *Nature* 418, 443 - 447 (2002)



Effect of NBD (IKKγ) peptide (Cat. No. IMG-2000) on human multiple myeloma cells.

NEMO binding domain (NBD) peptide inhibits constitutive NF-κB and induces cytotoxicity in human multiple myeloma cells. Untreated or NBD peptide-treated (100 μM; 12 hours). Red stain indicates the localization of p65, and blue stain indicates nucleus. Original magnification, x 200.

Please inquire about pricing and larger quantities, or go to our website at www.imgenex.com

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