

Pyroptosis/Caspase-1 Assay Kit

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CATALOG #

AAA12389

PRODUCT SYNONYMS

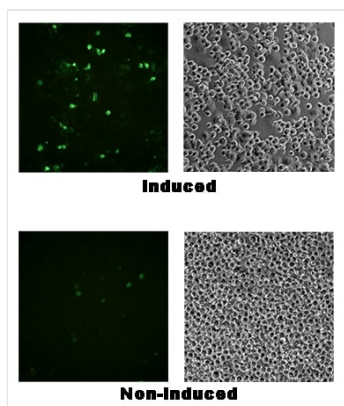
Pyroptosis/Caspase-1; Pyroptosis/Caspase-1 Assay Kit; Pyroptosis/Caspase-1 assay kit

PRODUCT SPECIFICATIONS

Preparation and Storage

Store at ≤ -20 degree C for Nigericin component, 2-8 degree C for remaining kit components.

IMAGES



Application Data

THP-1 suspension cells were treated with PMA (5 ng/mL) to induce differentiation into macrophages. After 48 hours, PMA was removed and replaced with fresh medium that was spiked with LPS (10 ng/mL) to induce caspase-1 activation. After 2 hours, cells were stained with FAM-YVAD-FMK for 1 hour, washed, and examined under a fluorescence microscope. The top images reveal many experimental cells, several of which fluoresce green, therefore they have active caspase-1. The lower right image reveals many control cells in the field of view, however the corresponding fluorescence image is dark (lower left); few of these cells have active caspase-1. Data courtesy of Dr. Brian Lee, ICT.

ADDITIONAL INFORMATION

Related Product Information for Pyroptosis/Caspase-1 assay kit	Background: Exposure of inflammatory effector cells like monocytes and macrophages to pathogen-associated molecular patterns (PAMPS), such as viral or bacterial DNA or RNA and bacterial cell wall components like LPS, will typically trigger conformational changes in NACHT leucine-rich repeat protein family (NLRP) proteins^{1,2}. Of the inflammasomes, the NLRP3 inflammasome is the most studied. Activation of the NLRP3 inflammasome follows exposure to PAMPS (first signal) and ATP (second signal) (Figure 1). This leads to oligomerization and assembly of a high molecular weight (~700 kDa) multimeric inflammasome complex, which leads to the conversion of pro-caspase-1 into the catalytically active form. Inflammatory caspases, such as caspase-1, or interleukin-converting enzyme, play a central role in innate immunity by recognizing foreign danger signals and initiating a two-fold response. First, caspase-1, proteolytically converts the proforms of the two important pro-inflammatory cytokines, interleukin 1beta (IL-1beta) and interleukin 18 (IL-18), into their active forms, which are secreted. Second, caspase-1 or caspase-11 triggers a form of lytic, programmed cell death known as pyroptosis^{3,4}. Pyroptosis is a highly inflammatory form of programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial immune response. This pathway is distinct from apoptotic cell death in that it results in plasma-membrane rupture and the release of pro-inflammatory cytokines; infected cells eventually swell, burst, and die. This, in turn, attracts other immune cells to fight the infection, leading to inflammation of the tissue, and, in a functional response, rapid clearance of bacterial or viral infections. Pyroptosis/Caspase-1 Assay Kit utilizes our popular FLICA technology to detect caspase-1 activation. FLICA probes are cell permeant noncytotoxic Fluorescent Labeled Inhibitors of CASpases that covalently bind with active caspase enzymes^{5,6}. The kit contains the caspase-1 inhibitor reagent FAM-YVAD-FMK, which has the preferred binding sequence for caspase-1, Tyr-Val-Ala-Asp (YVAD)⁷. This preferred caspase-1 binding sequence is labeled with a green fluorescent carboxyfluorescein (FAM) dye and linked to a fluoromethyl ketone (FMK) reactive entity. Caspase-1 will not cleave the FLICA inhibitor probe; instead, it forms an irreversible covalent bond with the FMK group on the reagent and becomes inhibited from further enzymatic activity. To use FLICA, add it directly to the cell culture medium, incubate, and wash. FLICA is cell-permeant and will efficiently diffuse in and out of all cells. If there is an active caspase-1 enzyme inside the cell, it will covalently bind with FAM-YVAD-FMK and retain the green fluorescent signal within the cell. Unbound FLICA will diffuse out of the cell during the subsequent wash steps. Therefore, positive cells will retain a higher concentration of FLICA and fluoresce brighter than negative cells. There is no interference from pro-caspases or inactive forms of the enzymes. After labeling with FLICA, cells can be counter-stained with other reagents and fixed or frozen. Cells labeled with FAM-YVAD-FMK can be counter-stained with reagents such as the red live/dead stains Propidium Iodide and 7-AAD. Nuclear morphology may be concurrently observed using Hoechst 33342 (included in the kit), a blue DNA-binding dye. Cells can be viewed through a fluorescence microscope (Figures 2 and 4), or the fluorescence intensity can be quantified using a fluorescence plate reader (Figure 3), or flow cytometer (Figure 5). FAM-FLICA optimally excites at 488-492 nm and has a peak emission at 515-535 nm. Nigericin, a potent microbial toxin derived from <i>Streptomyces hygroscopicus</i>, acts as a potassium ionophore, inducing a net decrease in intracellular level of potassium which is crucial for oligomerization of the NLRP3 inflammasome and activation of caspase-1. Nigericin requires signaling through pannexin-1 to induce caspase-1 activation and IL-1beta processing and release. Nigericin is included in this kit as a positive control. It has been shown to generate a robust caspase-1 activation response in various cell lines, including Jurkat (Figure 5) and THP-1 cells. FLICA is for research use only. Not for use in diagnostic procedures.
Product Categories/Family for Pyroptosis/Caspase-1 assay kit	Caspase-1; Caspases; Pyroptosis

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/pyroptosis-caspase-1/12389>