

Necrosis vs Apoptosis Assay Kit

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CATALOG #
AAA52736

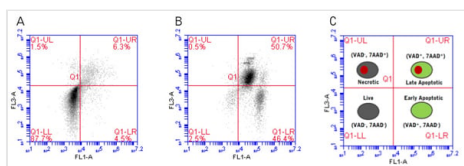
PRODUCT SYNONYMS

Necrosis vs Apoptosis; Necrosis vs Apoptosis Assay Kit; Necrosis vs Apoptosis assay kit

PRODUCT SPECIFICATIONS

Samples	Cell culture
Excitation/Emission	FAM-FLICA - 492 nm / 529 nm; 7-AAD - 546 nm / 647 nm
Reagent	FAM-VAD-FMK
Preparation and Storage	Store at 2-8 degree C.

IMAGES



Application Data

Flow cytometry analysis of Jurkat suspension cells to quantify four populations. (A) Cells were treated with a placebo (non-induced treatment with DMSO). (B) Cells were treated with 1 μ M staurosporine for 4 hours to induce apoptosis via caspase activity. Cells were then dually stained with FAM-FLICA and 7-AAD, and analyzed using an Accuri C6 flow cytometer. FAM-FLICA was analyzed on FL-1 and 7-AAD was analyzed on FL-3. The key is shown in (C). Live, unstained cells do not fluoresce (lower left quadrant). Early apoptotic cells fluoresce green with FAM-FLICA. Dually stained green and red fluorescing cells represent the population of cells in mid to late apoptosis (these cells have active caspase enzymes and compromised cell membranes). Necrotic cells fluoresce red. In the non-induced population (A), only 9.8% of cells were apoptotic (LR: 4.5% + UR: 6.3%), compared with 97.1% of the induced population (B; LR: 46.4% + UR: 50.7%).

ADDITIONAL INFORMATION

Related Product Information for Necrosis vs Apoptosis assay kit	Background: Assessment of cellular cytotoxicity levels associated with cytolytic activity of T lymphocytes or natural killer cells is an important aspect of immunobiology related research¹. Early assays developed to assess cytolytic activity utilized the release of ⁵¹Cr from membrane compromised target cells, which were passively loaded with this radioactive indicator prior to exposure to the cytotoxic agent or cells². A major draw-back to these ⁵¹Cr release assays is their reliance on the use of a radioactive isotope with its associated disposal and safe handling issues. Additionally, chromium uptake methods suffer from variabilities in target cell preloading inconsistencies as well the tendency to spontaneously release the chromium isotope into the media in the absence of any cytotoxicity stimulus³. Assessing potential cytotoxicity properties of chemical and biological agents is a mandatory requirement for the safe distribution of pharmaceuticals, vaccines, or additives associated with food product formulations. Early identification of unintended drug, vaccine, or chemical associated cytotoxicity properties is always an early priority of initial FDA approval testing protocols. With cellular cytotoxicity assessment playing a central role in countless research and environmental safety studies, there is an ever present need for simple, straightforward analysis methods like the Necrosis vs Apoptosis Assay. The Necrosis vs Apoptosis Assay simultaneously detects both apoptosis associated cytotoxicity events as well as cell death due to necrosis. Apoptotic cells are identified using Fluorescent Labeled Inhibitor of Caspases (FLICA) reagent probe⁴⁻⁷. The FAM-FLICA probe covalently binds to active caspase enzymes, which are up-regulated during apoptosis, thus clearly labeling apoptotic cells for subsequent analysis^{8,9}. Non-apoptotic cells will not contain the active caspase enzymes required for FAM-FLICA to remain covalently bound within the cell structure. Loss of the integrity of the cell membrane, indicative of necrosis or late stage apoptosis, is detected using the vital staining dye, 7-aminoactinomycin D (7-AAD), a red fluorescing live/dead stain. This dye easily penetrates cell membrane-compromised cells, binding tightly to GC rich regions of the DNA¹⁰⁻¹⁴. 7-AAD will not label cells in early stages of apoptosis, as their cell membranes are still intact and are capable of excluding 7-AAD. As caspases are active during early apoptosis, combining FAM-FLICA with 7-AAD can provide a more detailed picture of the overall health of the cell population by revealing the percentage of cells that are 7-AAD-negative (membrane intact live cells) and yet FAM-FLICA positive (apoptotic) (Figures 3-6). FAM-FLICA probes optimally excite at 488-492 nm with maximal emission at 515-535 nm. The vital staining dye 7-AAD optimally excites at 546 nm and emits at 647 nm. This significant difference in fluorescence emission wavelength between the green FAM (carboxyfluorescein) label on the FAM-FLICA probe and the red 7-AAD vital dye simplifies flow cytometer gating and compensation. The FAM-FLICA probe (apoptosis) is monitored on the FL-1 channel, while 7-AAD (necrosis) is monitored on FL-3. Combining the use of FAM-FLICA apoptosis detection probe with a membrane integrity dye like 7-AAD makes it easy to distinguish between necrosis and apoptosis within a single sample.
Product Categories/Family for Necrosis vs Apoptosis assay kit	Apoptosis ; Caspases

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/necrosis-vs-apoptosis/52736>