

Quantitative Measurements of Apoptosis using the NovoCyte Flow Cytometer



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Introduction

Apoptosis, or programmed cell death, is an active process where cells regulate how they die by triggering specific pathways that cause cells to shrink, condense, and eventually be cleared by phagocytosis. This is in contrast to necrotic cell death where cells die uncontrollably due to external factors, leading to detrimental effects such as the activation of an immune response. Apoptotic cells that die in an orderly fashion cause limited disruption to nearby cells and tissue.

Apoptosis is a multistep process that can be interrogated at different stages. Early in apoptosis, the mitochondria lose membrane potential and are unable to function properly, leading to cytochrome c release and the activation of caspases. The early stage of apoptosis also involves phosphatidylserine (PS) translocation from the inner cellular membrane to the outer leaflet, exposing it to the extracellular environment. During later stages of apoptosis, cellular membranes become permeable, compromising their integrity and selective transport of materials.

Materials and methods

To demonstrate the flexibility and versatility of the Agilent NovoCyte flow cytometer system in assessing apoptosis, three assays were carried out to measure apoptosis in response to staurosporine, a broad kinase inhibitor known to induce apoptosis in many cell types. The first assay uses annexin V to bind exposed PS on cell membranes for labeling of early apoptotic cells and the DNA binding dye, 7-AAD, for labeling of late apoptotic cells. This assay shows that staurosporine induces apoptosis in both Jurkat T cells and HeLa cells (Figure 1), and can distinguish cells at the early and late stages of apoptosis.

The second assay makes use of the fluorescent dye JC-1 to measure the loss of mitochondrial membrane potential in early apoptotic cells. JC-1 is cell-permeable and aggregates inside mitochondria while membrane potential is maintained, emitting fluorescence in the PE channel. When the membrane potential is lost, JC-1 will not localize to mitochondria and will reside in the cytoplasm in its monomeric form, emitting fluorescence in the FITC channel. The ratio of PE to FITC fluorescence can therefore represent changes in the mitochondria membrane potential. As shown in Figure 2, compared to untreated controls, Jurkat T cells treated with staurosporine have reduced mitochondrial membrane

potential. This potential continuously decreases over time up to 6 hours after treatment. The response is similar to that observed on cells treated with a known mitochondrial membrane depolarizing agent, FCCP.

The third assay quantifies caspase activity. Probe molecules containing a cleavage site specific for caspases 3 and 7 are added to cells. When cleaved by these activated caspases in early apoptotic cells, the probe molecules enter the nucleus, bind to DNA, and fluoresce. This enables a direct readout of activated caspases in cells. As shown in Figure 3, staurosporine-treated Jurkat T cells have more caspase activity than the untreated, control cells.

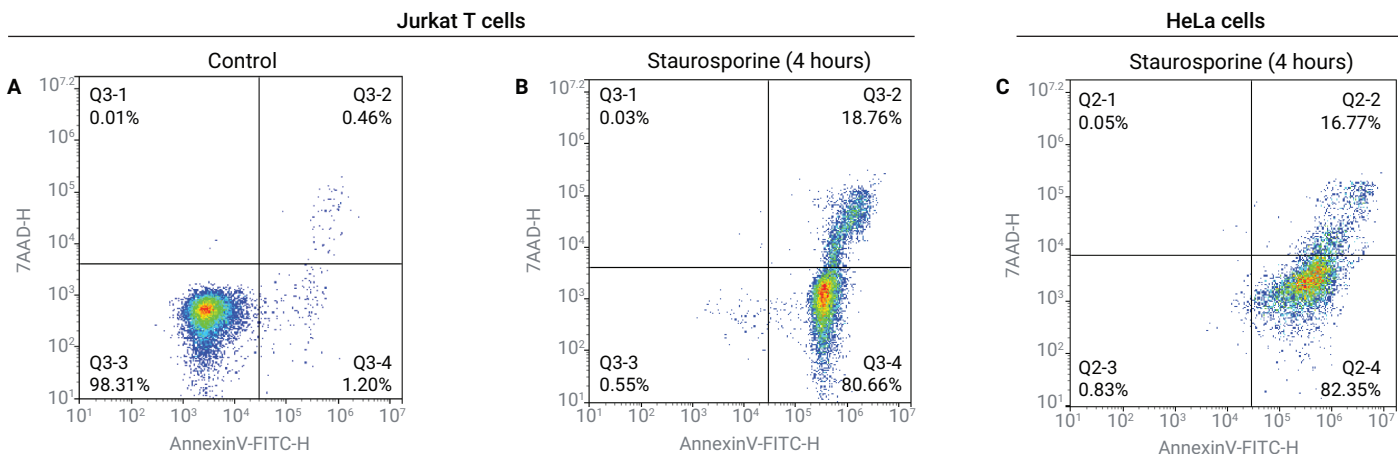


Figure 1. Detection of early apoptosis using annexin V/7-AAD in staurosporine-treated cells. Jurkat T cells were subjected to vehicle control (A) or 2 μ M staurosporine (B), and HeLa cells were subjected to 2 μ M staurosporine (C) for 4 hours. Following treatment, cells were analyzed for early and late apoptotic cells by staining for phosphatidylserine with annexin V-FITC and with the DNA binding dye 7-AAD (BioLegend), followed by flow cytometric analysis. Cells were classified as early apoptotic with annexin V-only positive staining, and late apoptotic (or dead) cells with annexin V and 7-AAD positive staining.

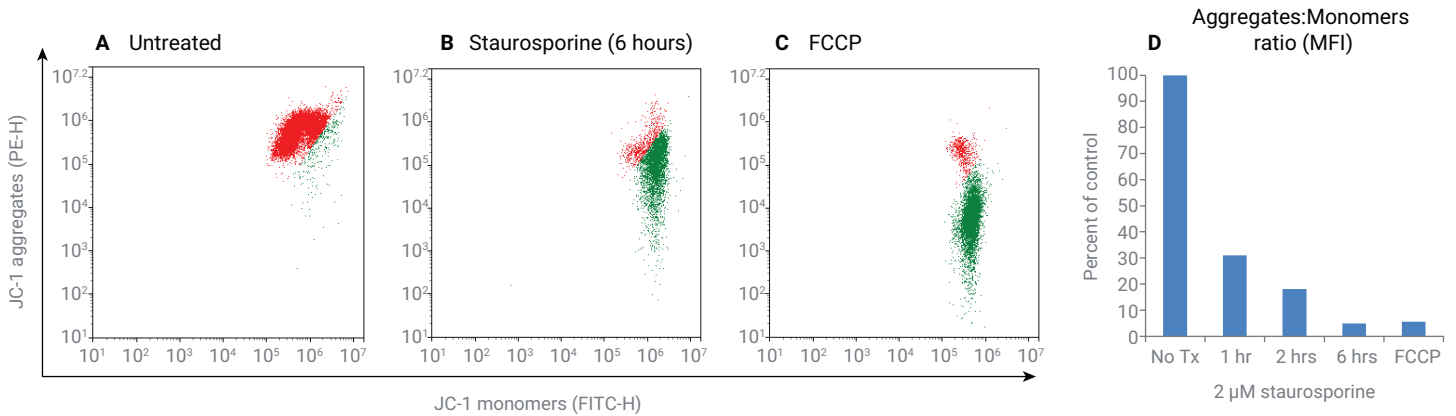


Figure 2. Staurosporine compromises mitochondrial membrane potential in Jurkat T cells. Jurkat T cells were treated with vehicle (A), 2 μ M staurosporine for 6 hours (B), or with FCCP (C). JC-1 dye (Life Technologies) was added to the cells to measure mitochondrial membrane potential. In healthy cells, the dye is localized to the mitochondria and forms aggregates, emitting fluorescence in the PE channel. In cells where the mitochondrial membrane potential has been compromised, the dye localizes to the cytoplasm as monomers, emitting fluorescence detectable in the FITC channel. The effect of compound treatment of the cells can be evaluated based on the ratio of PE to FITC fluorescence. (D) A bar graph representing the ratio for Jurkat T cells at different time points following treatment with staurosporine. All treated cells (including FCCP positive control) show a lower ratio than the vehicle-treated control.

Results and discussion

The three assays shown here offer quantitative, robust, and reproducible methods for measuring populations of cells undergoing apoptosis at different stages. These apoptotic assays are easy to perform with the NovoCyte flow cytometer due to its automatic compensation capability and wide dynamic range of fluorescence detection, eliminating the need for PMT voltage adjustments.

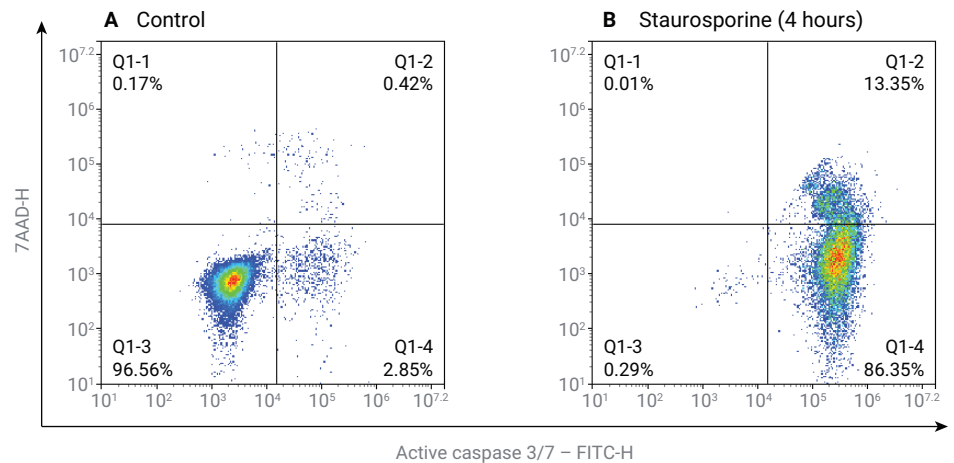


Figure 3. Detection of caspases 3 and 7 activity in Jurkat T cells treated with staurosporine. Jurkat T cells were treated with 2 μ M staurosporine for less than 1 hour (A) or 4 hours (B) and stained with cell-permeable, DEVD-conjugated fluorescent nucleic acid binding dye (Life Technologies) and 7-AAD dye (BioLegend). Untreated control cells show minimal caspase activity. After 4 hours of treatment with staurosporine, cells exhibit an increase in caspases 3 and 7 activity (99.7%).

References

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