

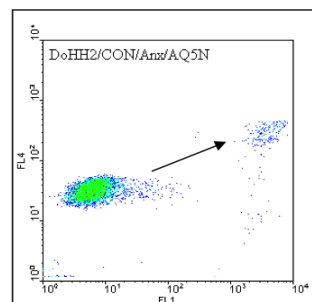
APOPTRAK™ in FC-Based Apoptosis Assays

Far-Red Fluorescent Dye Reporting Cell Integrity

1. LIVE CELL MARKING AND CELL DEATH SCORING

BACKGROUND

In apoptosis an ordered series of events leads to destruction of a single cell. The changes that occur can be assayed through DNA fragmentation, caspase activity (using permeant substrates e.g. FLICA™ probes), mitochondrial membrane potential (e.g. using TMRM), cytochrome C release, plasma membrane inversion (using Annexin V) and permeabilization being common choices. Plasma membrane permeabilization is a late hallmark of apoptosis identified with a viability probe and relative frequencies of intact, compromised and dead cells in a test sample are important monitors of sample quality and the mode of cell damage/death.



WHAT IS THE PROBLEM?

There are a variety of fluorescent probes available to monitor cell health, the activation of cell death pathways and linked shifts in the biophysical and bioenergetic properties of the cell. A well-documented change during cell death, including the later phases of apoptosis, is the loss of plasma membrane integrity. Such cells lose their ability to resist the penetration of fluorescent dyes into one or more cellular compartments. A favoured approach for flow cytometry is to focus on this late stage event to permit a rapid scoring or exclusion by 'gating out' of dead cells. The analysis of subpopulations undergoing cell death can be somewhat subjective and dependent on the properties of the dye used. Typically a non-permeant nucleic acid targeting dye is used to detect this loss of membrane integrity and the instrument set up to achieve the best differentiation between non-labelled 'intact' cells and labeled 'dead' cells. A problem arises when there is a need to positively mark live cells in addition to the compromised cells and monitor them in the same fluorescence channel. The advantage of a differential positive labelling of subpopulations is that it allows other parameters, such as changes in light scatter, to be co-assessed reliably as cell-based events. The properties of APOPTRAK™ offer a solution to this problem.

HOW DOES APOPTRAK™ HELP?

APOPTRAK™ is a low toxicity dye that shares the spectral properties of DRAQ5™ and DRAQ7™ but with a reduced ability to bind to cellular DNA. Being charge neutral APOPTRAK™ is capable of entering viable cells at low but detectable rates to primarily occupy cytoplasmic compartments. This property provides a non-complex far-red positive fluorescence signal primarily determined by the intact cell size. The low quantum yield and lack of fluorescence enhancement means that optimal fluorescence excitation is achieved with He-Ne (633 nm) to Kr-Ar (647 nm) lasers. APOPTRAK™'s low far-red fluorescence signal in 'intact/live' cells increases when there is greater dye access upon the disruption of the plasma membrane. Residual DNA binding capacity of APOPTRAK™ permits the detection of the nuclear fragments during cell break-up. This balance of properties means that APOPTRAK™ can be readily applied in cell death assay protocols. For example, is APOPTRAK™ is spectrally compatible with Annexin V-FITC and rhodamine-derived probes.

APOPTRAK™ is stored in a ready-to-use aqueous format with a very long shelf-life.

APOPTRAK™ Product Features:

- ❖ positive discrimination between intact cells, membrane-compromised cells, cellular debris
- ❖ optimally excited by red laser lines
- ❖ far-red fluorescence compatible with visible range fluorochromes, including GFP/FITC + R-PE
- ❖ validated for use in Annexin V binding assay protocols
- ❖ water-soluble: ready-to-use from the fridge



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