DRAQ7™ in Image-Based Apoptosis Assays

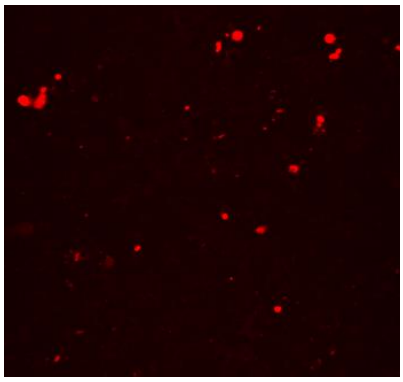
Far-Red Fluorescent Live-Cell Impermeant DNA Dye

DR7.APPNOTE: IMAGE APOPTOSIS 002 100523

1. IDENTIFYING MEMBRANE-PERMEABILIZED CELLS IN APOPTOSIS ASSAYS

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, BAX translocation, 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. This is combined with other apoptotic features to give a temporal context and to identify cells permeabilized by non-apoptotic means.



WHAT IS THE PROBLEM?

To achieve such analysis by fluorescence-based imaging, traditional fluorescent viability dyes like propidium iodide (PI) or DAPI have been used. However, each has challenges associated with it. PI progressively equilibrates with intact cells resulting in these cells becoming weakly positive making it difficult to reliably discriminate negative from positive cells. Importantly, PI's fluorescence overlaps with brightly fluorescing and common antibody conjugates and with TMRM and JC-1, commonly used mitochondrial membrane potential probes. DAPI is UV-/violet-excited and cannot be combined with UV-excited Hoechst 33342 probe commonly used as an "all-event" marker in apoptosis and cell health assays.

Photo-switching of DAPI and photo-bleaching of PI as well as evidence of semi-permeance make these DNA binding agents incompatible with long-term assays. Also, UV excitation is not always available on fluorescence microscopes. Typically these agents need to be prepared fresh from hard compound and cannot be stored long-term in a ready-to-use solution.

HOW DOES DRAQ7 HELP?

As a far-red DNA-binding viability dye DRAQ7™ removes the problem of spectral overlap with TMRM (PI) and UV-excited dyes like monochlorbimane, a Glu-SH probe, and Hoechst 33342 (DAPI) which can be used together with DRAQ7™. For example, DRAQ7™ can be combined with Hoechst 33342, Annexin V-FITC, TMRM - a useful and temporally-relevant combination of parameters. DRAQ7™ is extremely chemically and photo-stable. In most apoptosis assays DRAQ7™ is an ideal replacement for PI, due to its spectral and practical advantages.

Uniquely, DRAQ7™ has proved compatible with long-term, real-time cell health assays. This means that DRAQ7™ can be added at any time and slides or wells imaged sequentially and non-destructively to determine the temporal development of the apoptotic events in the assay system.

DRAQ7™ is stored in a ready-to-use aqueous format with a very long shelf-life. It is convenient and easy to use.

DRAQ7™ Product Features:

- far-red fluorescing cell impermeant dsDNA probe
- rapidly and clearly labels only membrane-compromised cells
- compatible with UV-excited, fluorescein- and rhodamine-based chromophores
- validated for long-term, real-time apoptosis monitoring
- water-soluble; ready-to-use from the fridge

For a full price list and further information see **www.biostatus.com** or contact us at:
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