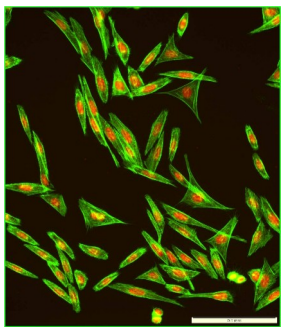
BIOSTATUS

DRAQ5™ in Immunofluorescence (IF)

Far-Red Fluorescent Live-Cell Permeant DNA Dye

1. NUCLEAR COUNTERSTAINING

BACKGROUND



Immunofluorescence (IF) microscopy involves the analysis of adherent cells or tissue sections where samples have been fixed or preserved with formaldehyde (FA) or other fixative. Typically, they are then further permeabilized with a weak surfactant to make the cells porous to antibodies. In this way fluorescently-tagged antibodies can be used to label internal structures in adhered cells or thin tissue sections on a suitable optical surface such as a slide or microplate well. It is important to see the antibody staining of the cells in the context of individual cells and their internal structure as well as any tissue morphology. This is most readily aided by the use of a nuclear counterstain. This allows counting of individual cells, segmentation of nuclear and cytoplasmic compartments and, in tissues, more information on morphology. Fluorescence images can be overlaid with phase contrast or brightfield images to provide additional detail.

WHAT IS THE PROBLEM?

An ideal nuclear counterstain for IF microscopy should show good specificity for the nuclear compartment (dsDNA) with negligible non-specific staining of any other compartments. Propidium iodide has high promiscuity for RNA and does not allow segmentation of the nucleus. Also, it must combine with common buffers and fixatives used in IF and have low photo-bleaching to permit identification of weak antigens and enable review. Spectral properties should allow combination with visible-range chromophores to permit it to be a default reagent for IF. The red DNA dyes TOTO-3 and TOPRO-3 overlap with commonly used rhodamine-based chromophores. Similarly, it should not undergo photo-switching after illumination as has been seen with UV-excited DNA dyes DAPI and Hoechst, which occludes the GFP/FITC channel. It should work with the widest range of cells and tissues. Perhaps most importantly, it should not be UV-excited as this is often reserved for highest performance microscopes (with environmental control, confocal, 2-photon) and the majority of IF microscopy can be performed on more freely available widefield (or epifluorescence) microscopes. This avoids delays in analysing samples or indeed blocking the access to high-end systems for complex analyses. Except for Hoechst 33342, these are cell membrane-impermeant dyes and do not have utility for transfer to live cell experiments.

For ease of use, the ideal counterstain is in a ready-to-use aqueous format. The dyes above are often supplied in hard compound form and need to be initially resolved in DMSO solvent and have limited stability in solution.

HOW DOES DRAQ5™ HELP?

The far-red cell permeant DNA probe DRAQ5™ shows high specificity and stoichiometry for dsDNA to give crisp segmentation of nuclei and chromatin ultrastructure, with negligible non-specific binding in IF microscopy. DRAQ5™ is chemically robust in IF buffers and shows remarkably low photo-bleaching. The far-red emission means that it is widely compatible with visible-range chromophores and does not experience photo-switching. DRAQ5™ has been demonstrated on a plethora of mammalian cells and tissues. It allows use of non-UV equipped microscopes for the widest equipment access and is transferable to live-cell end-point imaging experiments. DRAQ5™ is supplied in a ready-to-use aqueous format and has excellent shelf-life.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapidly and stably labels nuclei in cells and tissues
- optimally excited by red laser lines, no UV requirement
- compatible with UV-excited and visible range chromophores
- water-soluble; ready-to-use from the fridge

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