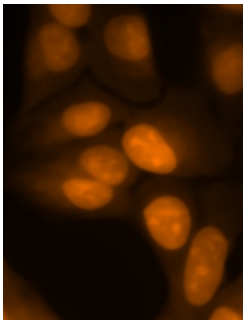
CyTRAK Orange™ in ImmunoFluorescence

Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

CO5.APPNOTE: IMMUNOFLUOR 001 130814

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. They are then 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 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 transmission 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 also for 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 red chromophores. Similarly, it should not undergo photo-switching after illumination as is the case with the UV-excited DNA dyes DAPI and Hoechst, which can occlude the heavily used 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 or 2-photon) and the majority of IF microscopy can be performed on the more freely available widefield (or epifluorescence) microscopes. This avoids delays in analysing samples or blocking 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 should be in a ready-to-use aqueous format. The dyes above are supplied in hard compound form and need to be initially resolved in DMSO solvent and have limited stability in solution.

HOW DOES CyTRAK Orange™ HELP?

Orange/red cell permeant DNA probe CyTRAK Orange™ shows high specificity for dsDNA to give good segmentation of nuclei and a useful differential cytoplasmic signal (with increased gain). CyTRAK Orange™ is chemically robust in IF buffers and shows very low photo-bleaching. Orange emission means it is widely compatible with UV-excited, GFP/FITC and red-excited (as it is not) chromophores and does not photo-switch. CyTRAK Orange™ has been demonstrated on a range of mammalian cells and tissues. Being blue/green excited it allows use of non-UV equipped microscopes for the widest equipment access and is transferable to live-cell imaging experiments. CyTRAK Orange™ is supplied in a ready-to-use aqueous format and has excellent shelf-life.

CyTRAK Orange™ Product Features:

- Orange-fluorescing cell-permeant dsDNA probe
- rapidly and clearly labels all nucleated cells (live or fixed)
- single-channel dual compartment (nucl:cyto) segmentation
- compatible with UV- & red-excited, & GFP/FITC dyes
- 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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