DRAQ*fx*™ in Immunofluorescence

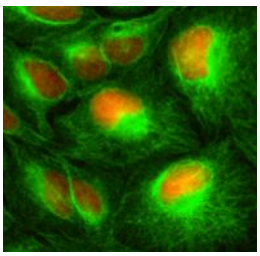
Dropper Bottle Far-Red Counterstain For FIXED Samples

DFX.APPNOTE: IMMUNOFLUOR 001 240517

1. NUCLEAR COUNTERSTAINING

BACKGROUND

Immunofluorescence* (IF) microscopy often involves the analysis of adherent cells or tissue sections where samples have been preserved with formaldehyde-/formalin-fixation (FF). Tissue samples can be paraffin–embedded (FFPE or snap-frozen. After sectioning they are processed to enable analysis, which might include dewaxing, rehydration, antigen retrieval and permeabilized with weak surfactant to make cells permeable to antibodies. Then, fluorescently-tagged antibodies can be used to label internal structures in adhered cells or thin tissue sections. It is important to view any antibody staining of cells in the context of an individual cell and its internal structure as well as any adjacent tissue morphology. This is aided by use of a nuclear counterstain to allow cell counting, nuclear/cytoplasm segmentation and, in tissues, more information on morphology. Fluorescence images can also be overlaid with phase contrast or transmission images to provide additional detail.



WHAT IS THE PROBLEM?

An ideal IF counterstain should show nuclear compartment (dsDNA) specificity i.e. negligible non-specific staining elsewhere. Propidium iodide has high promiscuity for RNA limiting nuclear segmentation. 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 default use. Orange-red DNA dyes TOTO-3 and TOPRO-3 overlap with commonly used red chromophores. Similarly, it should not undergo photo-switching after illumination seen with the UV-excited DNA dyes DAPI and Hoechst occluding 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 high performance microscopes (with environmental control, confocal, 2-photon) when most IF microscopy can be done on more freely available widefield microscopes. This avoids delay in analysis or the blocking of access to high-end systems.

Ideally a counterstain should be ready-to-use. The dyes above are usually supplied in hard compound form requiring dissolution in DMSO and/or have limited stability and typically need storage in frozen aliquots.

HOW DOES DRAQ*fx*™ HELP?

DRAQ*fx*™ shows high specificity and stoichiometry for dsDNA to give crisp segmentation of nuclei and chromatin ultrastructure, with negligible non-specific binding in IF microscopy. DRAQ*fx*™ is chemically robust in IF buffers and shows remarkably low photo-bleaching. Far-red emission makes it widely compatible with visible-range chromophores and does not undergo photo-switching. DRAQ*fx*™ is specifically designed for counterstaining fixed cell preparations and tissue sections. It allows use of non-UV equipped microscopes for the widest equipment access.

DRAQ*fx*™ FIX & GO is a ready-to-use aqueous dropper bottle format of DRAQ*fx*™ for ultimate convenience without compromising its performance as a fixed cell counterstain. It has excellent benchtop shelf-life.

DRAQ*fx*™ FIX & GO Product Features:

- far-red fluorescing DNA counterstain
- convenient, ready-to-use dropper bottle
- rapidly labels nuclei in fixed cells and tissues
- optimally excited by red (& orange) laser lines
- combines with UV-excited & vis. range fluors

For a full price list and further information see **www.biostatus.com** or contact us at:
BioStatus Limited
56a Charnwood Road, Shepshed, Leicestershire LE12 9NP United Kingdom
T +44 1509 558 163 | **F** +44 1509 651 061 | **E** enquiry@biostatus.com | **W** www.biostatus.com