DRAQ5™ in In-Cell Western Screens

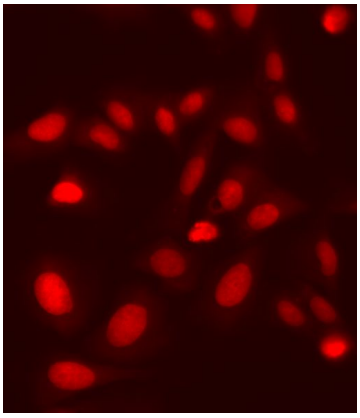
Far-Red Fluorescent Live-Cell Permeant DNA Dye

DR5.APPNOTE: ICW SCREEN 001 050814

1. IN-CELL WESTERN SCREENS

BACKGROUND

In-cell western assays involve cells being seeded into wells and exposed to the treatment of interest. After treatment the cells are fixed and permeabilised to allow intracellular access of a fluorescently-tagged antibody to label and quantify the protein of interest in all the cells across the well. Although great care is taken to make sure that the number of cells in each well is equal there are inevitably differences that will impact the statistical robustness of data seen across different treatments and dose dilutions.



The most obvious means of normalising the signal from each well is to relate the number of cells to the signal from a fluorescing stoichiometric DNA probe binding the DNA in all the cells present. The resulting aggregate signal is directly proportional to the number of cells and can be used to normalise the protein expression appropriately.

WHAT IS THE PROBLEM?

The majority of instruments used for in-cell westerns (e.g. LICOR Odyssey) only detect far-red and infra-red fluorescence. (Recently released instruments such as TTP Labtech's Acumen eX^3 and Mirrorball will allow combination of a wider selection of chromophores.) In addition, however, the DNA dye used needs to show high specificity and stoichiometry with nuclear dsDNA, low general non-specific binding, compatibility with physiological buffers and preferably in a standardised and ready-to-use aqueous preparation amenable to high throughput applications. Since microplates may need to be read more than once it is also important that the DNA dye does not rapidly photo-bleach and does not photo-switch after illumination. Blue-excited Propidium iodide is inappropriate due to its high promiscuity in binding RNA and broad spectral overlap with R-PE. DAPI and Hoechst 33342 have been shown to photo-switch into the green emission channel used for FITC/GFP and require UV excitation. Red fluorescing DNA dyes such as TOTO-3 and TO-PRO-3 have not been adopted as their spectral properties have not permitted detection and as with the others DNA dyes here need to be resolved from hard compound with DMSO, having limited storage in aqueous solution.

HOW DOES DRAQ5 HELP?

As a far-red fluorescing DNA probe DRAQ5™ has ideal spectral characteristics for the LICOR Odyssey platform. Additionally, it is compatible with most visible-range chromophores, becoming the default reagent in such assays. Importantly, DRAQ5™ has been demonstrated to have stable and stoichiometric binding to dsDNA, thereby providing good linearity for normalisation of protein signals well-to-well across a microtiterplate. DRAQ5™ is remarkably photo-stable, is chemically compatible with physiological buffers and is provided in a standardised and convenient ready-to-use aqueous formulation. It has been demonstrated in high throughput applications, utilising in-cell western screens for drug discovery programs.

DRAQ5™ Product Features:

- far-red fluorescing cell permeant dsDNA probe
- rapidly and stoichiometrically labels all nucleated cells
- excited optimally by red wavelengths (Ex max 646 nm)
- compatible with Horizon BV / BUV, FITC & R-PE 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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