Use of DNA-specific anthraquinone dyes to directly reveal cytoplasmic and nuclear boundaries in live and fixed cells

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Abstract

Image-based high-content screening assays, demand solutions for image segmentation and cellular compartment encoding to track critical events - for example those presented by GFP reporters within cell cycle tracking and GPCR translocation assays. We have designed nuclear and cytoplasm discriminator CyTRAK™ probes - spectrally compatible with all GFP reporter variants offering new solutions in cytometry.

At their most fundamental they provide a convenient fluorescent emission signature which is spectrally separated from the commonly used reporter proteins (e.g. EGFP, YFP, mRFP) and fluorescent tags such as Alexafluor 488, fluorescein and Cy2. Additionally, they do not excite in the UV and thus avoid the complications of compound UV-autofluorescence in drug discovery whilst limiting the impact of background sample autofluorescence. They provide a convenient means of stoichiometrically labeling cell nuclei in live cells without the aid of DMSO and can equally be used for fixed cells. Further developments have permitted the simultaneous and differential labeling of both nuclear and cytoplasmic compartments in live or fixed cells to render the cell boundaries which may be beneficial for quantitative expression measurements and in cell-cell interactions.

Examples of CyTRAK™ probes will be shown on the context of HCS imaging platforms, confocal and epifluorescence microscopy and flow cytometry.

Binding & Labelling properties

Compartments segmentation

- Label live or fixed cells - therefore suitable for a wide range of cell-based assays
- Use when there is a need to discriminate cell compartments - nucleus versus cytoplasm
- Easy to implement using conventional image analysis algorithms and most flow cytometry formats
- Suitable for enhanced GPCR assays where whole cell demarkation is required as well as cell nucleus
- Compatible with many HCS formats • many cell models • many GFP variants

Application on HCS platforms

Figure 1:
Application on HCS platforms

Figure 2:

Figure 3:

Figure 4:

Figure 5:

Figure 6:

Figure 7:

Figure 8:

The ability to reliably label intact cells has been successfully applied in homogeneous in-cell western assays. The resulting signal allows for the normalisation of reporter signal against the cell number in a well (DRAQ5, In-Cell Western™ assays, LI-COR).

Evotec Technologies' Opera

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