CyTRAK™ probes: novel nuclear and cytoplasm discriminators compatible with GFP-based HCS and HTS assays

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Why CyTRAK™ probes ?

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 variants of GFP-reporters offering new solutions in cytometry.

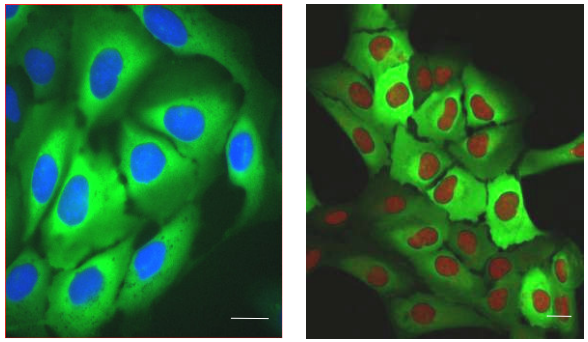


Figure 1: The GFP (green) compatibility of DRAQ5™ (left panel, blue nuclei) and CyTRAK Orange™ (right panel, red nuclei) provides rapid one-step cell feature discrimination in both live- and fixed-cell assays. Bar is 10 µm.

DRAQ5™

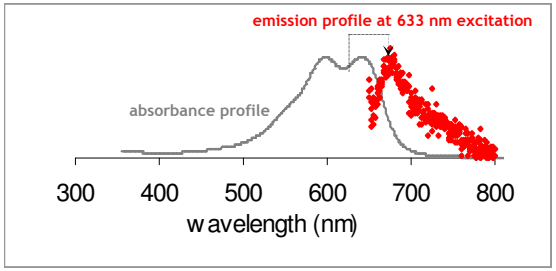
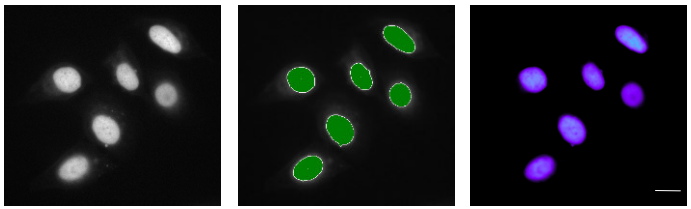


Figure 2: The DRAQ5™ far-red fluorescent probe (excitation 633 nm/emission 670 nm) is a rapid, no wash, live-cell nuclear label.



Step 1: Label cells with (5 µM) DRAQ5™ for 3-5 mins

Step 2: Single threshold value for classification of nuclear compartment

Step 3: No residual signal providing a clean nucleus identification only

Figure 3: DRAQ5™ is a high affinity DNA probe which ensures a robust identification of the nuclear compartment and is compatible with all image analysis algorithms for cell counting, and those designed to track receptor internalization and translocation. Bar is 10 µm.

CyTRAK Orange™

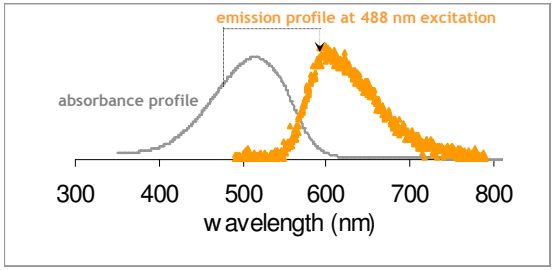
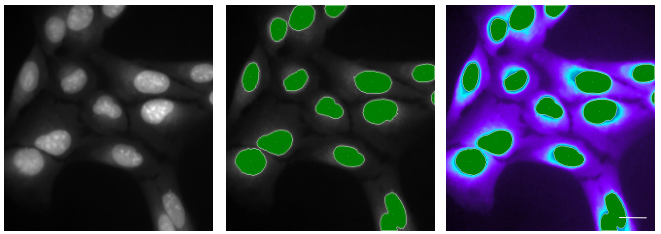


Figure 4: CyTRAK Orange™ (excitation 488 nm/emission 615 nm) is a rapid, no wash, live-cell cytoplasm and nuclear label, hence revealing the outline of the entire living cell



Step 1: Label cells with 5 µM CyTRAK Orange™ for 20-30 mins

Step 2: High intensity threshold value for classification of nuclear compartment

Step 3: Second lower intensity threshold to detect cell edge.

Figure 5: CyTRAK Orange™ rapidly enters live cells to intensity discriminate between nuclear and cytoplasmic compartments. As a dual compartment label this offers new opportunities for live cell-based assays where cell location, cell perimeter, cell shape and cell spread parameters can be used to define the assay at the single cell level. Bar is 10 µm.

The key benefits of CyTRAK™ probes for high-content screening

- 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
- Enhances GPCR assays where whole cell demarkation is required as well as cell nucleus
- Compatible with many HCS formats ▪ many cell models ▪ many GFP variants

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