PRODUCT INFORMATION & DYE TECHNICAL DATA

CyTRAK Orange™

NBP2-81127

For research use only.
Not for diagnostic or therapeutic procedures.

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CyTRAK Orange ™ TECHNICAL DATA

PRODUCT: CyTRAK Orange ™
CATALOG NUMBER: NBP2-81127

PRESENTATION: aqueous solution.
STORAGE: store at 2-8 °C. DO NOT FREEZE

DESCRIPTION:
CyTRAK Orange ™ is a novel orange fluorescent dye related to DRAQ5™ that stains both nucleus and cytoplasm with differential intensity (See fig. 3). It is water- soluble and membrane permeant and can be used in LIVE or fixed cells in combination with other common fluorophores, especially GFP fusions, FITC-labelled antibodies and far-red dyes. It is compatible with common cytometry and microscopy instruments.

The promiscuity of staining of the cytoplasm in addition to the nucleus allows definition of cells which are arrested / senescent based on increased CyTRAK Orange ™ fluorescence signals compared to healthy cells, using flow cytometry. Further, the secondary cytoplasmic signal is clearly weaker than the nuclear signal allowing automated segmentation of the two cellular compartments for high content image-based assays.

CyTRAK Orange ™ has been used to stain HUVECs for an endothelial cell migration assay where cell exposure to the dye was 3 days (Mierke, et al., 2011).

APPLICATIONS:
- Flow Cytometry – live (or fixed)
  Nucleated cell gating (no lyse, no wash)
  Arrested/Senescent cell identification (no lyse, no wash)
- Fluorescence Microscopy - live (or fixed)
- HCS & Cell-Based Assay counterstaining
  Drug, RNAi, phenotypic screens, In-cell westerns
  - nucl:cyto segmentation

BEFORE STARTING:
Read the MSDS. Wear protective clothing, safety goggles and laboratory gloves. Check the concentration of CyTRAK Orange ™ stated on the vial label.

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:
Phosphate-Buffered Saline (PBS, without azide), culture medium, plastic-ware paraformaldehyde, Triton-X 100, Tween-20, antibodies, blocking solution.

NOTE: As with any cell-permeant DNA intercalating probe, CyTRAK Orange ™ may inhibit cell division in long-term assays and should be tested for any effect.

DETECTING CyTRAK Orange ™ SIGNALS: (see figs. 1 & 2)
Flow cytometry: CyTRAK Orange ™ can be excited by blue and green laser sources. It is not excited by red light. Detect using bandpass filters centred on 610 nm. CyTRAK Orange ™ marks nucleated cells for gating (see fig. 4). CyTRAK Orange ™ can be used to discriminate arrested or senescent cells based on increased brightness.
Microscopy / HCS Imaging Platform: CyTRAK Orange ™ is optimally excited using blue/green wavelengths. It is detected with filters centred on 610 nm. Segment nuclear and cytoplasmic compartments by first segmenting nuclei, then mask and use a watershed algorithm to detect cytoplasm. It can be used in multi-color HCS, for example, with AlexaFluor 350, GFP and AlexaFluor 647 (Sawada, et al., 2011).

*As no washing is required, CyTRAK Orange ™ is added last. *Use 200 µl per coverslip; 100 ul per 96-MTP well, 30 ul per 384-MTP well, 10 ul per 1536-MTP well.*

SPECTRAL CHARACTERISTICS:
Exλ_{max} 510 nm Emλ_{max} 610 nm

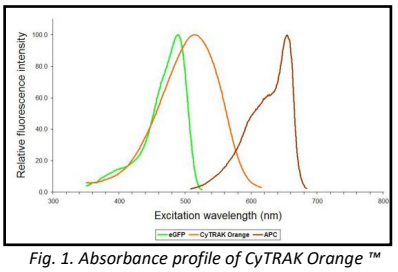

Fig. 1. Absorbance profile of CyTRAK Orange ™ compared to eGFP and APC

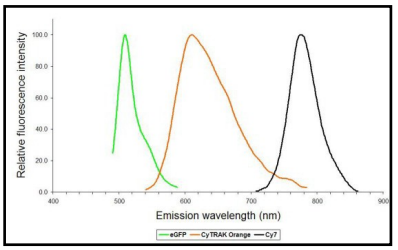

Fig. 2. Emission profile of CyTRAK Orange ™ compared to eGFP and Cy7

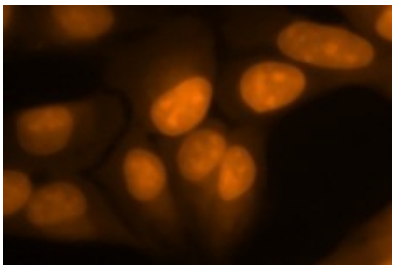
Fig. 3. CyTRAK Orange ™ counterstaining of fixed U2OS cells, showing differential cytoplasmic staining

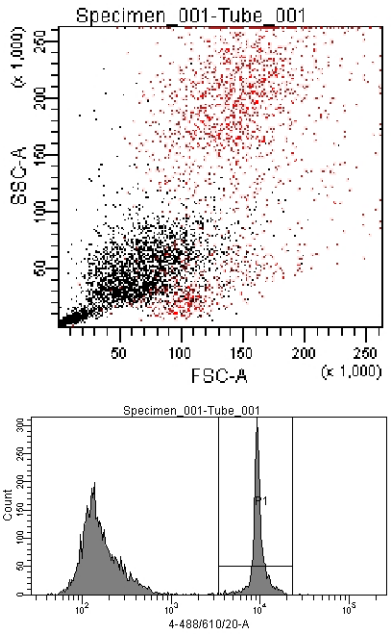

Fig. 4. Nucleated cell gating by CyTRAK Orange ™ intensity of intact, unlysed bone marrow with retained forward and side scatter characteristics

CyTRAK Orange ™ TECHNICAL DATA

EXAMPLE PROTOCOLS

PROTOCOL 1:
NUCLEATED AND SENESCENT/ARRESTED CELL GATING BY FLOW OR IMAGING CYTOMETRY

1. Prepare cells for staining with CyTRAK Orange ™: resuspend cells in appropriate buffer (PBS) at a concentration of ≤4 x 10^5 / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue sectionsize.
2. Add CyTRAK Orange ™ at 5-10 µM, final concentration. This will be an overlay for adherent cells / tissue sections, added to the well directly or in fresh medium following a wash step.
3. Gently mix, then incubate for 15-30 minutes at room temperature. n.b. protect from light. Staining is accelerated at 37ºC.
4. Analyze without further treatment / washing. n.b. analyze live cells within 2 h. CyTRAK Orange ™ stains live, fixed, permeabilized and dead cells.

PROTOCOL 2:
LIVE CELL COUNTERSTAINING FOR HCS IMAGING PLATFORM OR FLUORESCENCE MICROSCOPY

1. Wash and aspirate the slide or well.
2. Overlay cells with CyTRAK Orange ™ - final concentration 5 µM. See guideline volumes above*
3. Incubate for 15-30 minutes at room temperature. For time-lapsed assays (e.g. studying translocation of an EGFP tagged protein) CyTRAK Orange ™ may be added to the assay medium for the duration of the assay (typically 0.5 - 3 hr.) at 1 µM prior to any agonist / antagonist addition. n.b. protect from light. Staining is accelerated at 37ºC.
4. Analyze without further treatment / washing. CyTRAK Orange ™ stains intact, live, permeabilized and dead cells. False color CyTRAK Orange ™ images orange for simplicity.

PROTOCOL 3:
FIXED CELL COUNTERSTAINING FOR HCS IMAGING PLATFORM OR FLUORESCENCE MICROSCOPY

A. SEPARATE FIXATIVE & COUNTERSTAIN (e.g. when external (immuno-)fluorescent stains are applied):
1. Prepare separate working solutions of 4% formaldehyde (FA) and 5 µM CyTRAK Orange ™ in PBS.
2. Overlay slide or well with 4% FA. Incubate for 15-30 minutes at room temperature (RT) / 37ºC.
3. Gently aspirate FA, and wash with PBS.
4. Perform any necessary permeabilization, (immuno-)staining and blocking steps.
5. Wash with PBS to remove any residual Triton X-100, if used, and aspirate the sample. Overlay cells with CyTRAK Orange ™. See guideline volumes above*. Incubate for 10-20 minutes at RT. n.b. protect from light.
6. Analyze without further treatment / washing. False color CyTRAK Orange ™ images orange for simplicity.

B. COMBINED FIXATIVE & COUNTERSTAIN (e.g. when expressed fluorescent protein is the only analyte):
1. Prepare separate working solutions of 8% formaldehyde (FA) and 10 µM CyTRAK Orange ™ in PBS.
2. Overlay the slide or well with equal 0.5 volumes* of FA and CyTRAK Orange ™ solutions. **Alternatively**, pre-mix CyTRAK Orange ™ and FA working solutions to overlay. See guideline volumes above*
3. Incubate for 10-20 minutes at room temperature. n.b. protect from light.
4. Analyze without further treatment / washing. False color CyTRAK Orange ™ images in orange for simplicity.

KEY REFERENCES:

Edward, R. (2012) Meth. Enzymol. Vol. 505:23-45
Dimmick, I., et al. (2008) ISAC Conference; Poster
Sawada, J. et al. (2011) HCA Conference; Poster
Maiuri, L., et al. (2008) J. Immunol. 180: 7697
Mierke, C.T. (2011) J. Biol. Chem. 286: 40025

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