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1. Description

This product is for research use only.

Components	50 µL CyTRAK Orange™ Staining Solution or 200 µL CyTRAK Orange™ Staining Solution
Capacity	50 µL for 2.5×10^7 total cells, up to 50 tests or 200 µL for 1×10^8 total cells, up to 200 tests
Product format	The ready-to-use CyTRAK Orange Staining Solution is supplied as aqueous solution at a concentration of 5 mM.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

CyTRAK Orange is an orange cell permeable DNA stain which intercalates double-stranded DNA (dsDNA) of living or fixed cells stoichiometrically. In addition to the preferentially stained nucleus the cytoplasm is also stained weakly.

The fluorescence emission maximum for dsDNA-bound CyTRAK Orange is 610 nm. With a broad excitation band with a maximum at 510 nm it can be excited efficiently by blue (488 nm) lasers.

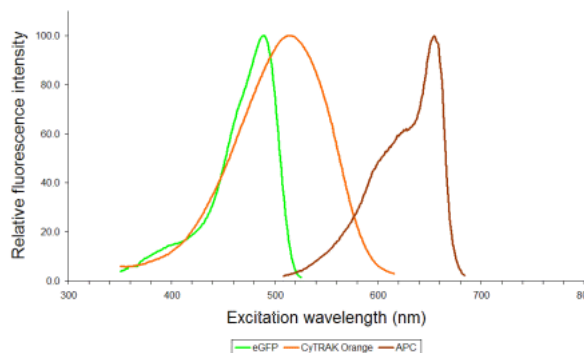


Figure 1: Absorbance profile of CyTRAK Orange compared to eGFP and APC profile of CyTRAK Orange compared to eGFP and APC.

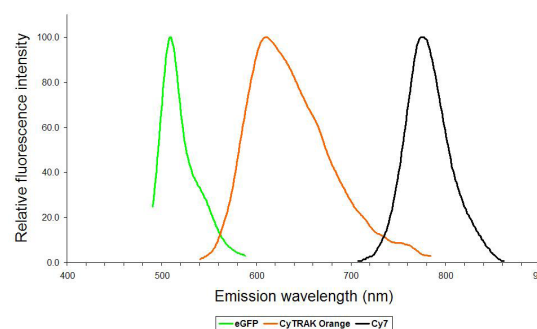


Figure 2: Emission profile of CyTRAK Orange compared to eGFP and Cy7.

1.2 Applications

- Enumeration of nucleated cells
- Nuclear counterstain in imaging and flow cytometry
- Arrested/senescent cell identification
- Nucl:cyto segmentation

1.3 Recommended dilution

It is recommended to use CyTRAK Orange Staining Solution at a final concentration of 5–10 µM (1:1000 to 1:500 dilution). Since applications vary, each investigator should titrate the reagent to obtain optimal results. Incubation times may vary typically between 15–30 minutes at temperatures between room temperature and 37 °C.

For nucleated cell enumeration add 1 µL of CyTRAK Orange Staining Solution to 5×10^5 cells in 1 mL buffer and incubate for 15 minutes at room temperature in the dark before analysis.

2. Examples of cell staining with the CyTRAK Orange Staining Solution

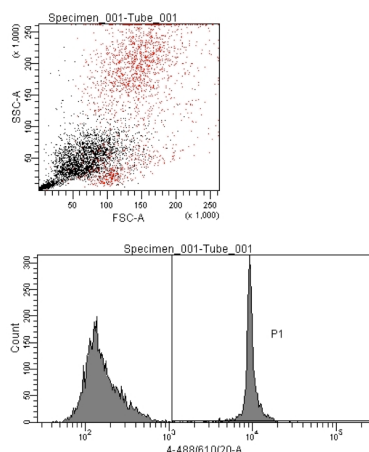


Figure 3: Nucleated cell gating by CyTRAK Orange intensity of intact, unlysed bone marrow with retained forward and side scatter characteristics.

3. References

1. Edward R. (2009) Use of DNA-specific anthraquinone dyes to directly reveal cytoplasmic and nuclear boundaries in live and fixed cells. *Mol. Cells* 27: 391–396.
2. Edward R. (2012) Red/far-red fluorescing DNA-specific anthraquinones for nucl:cyto segmentation and viability reporting in cell-based assays. *Methods Enzymol.* 505: 23–45.

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