

## LIVE CELL STAINING WITH CyTRAK Orange™ FOR NUCLEUS AND CYTOPLASM DISCRIMINATION

Appropriate for:

- **Wide-field camera-based imaging**
- **Confocal Laser Scanning Microscopy**

Reagents required:-

- **CyTRAK Orange™**
- Phosphate Buffered Saline (PBS, without sodium azide) or other culture medium

*CyTRAK Orange™ is intended for research purposes only*

- 
1. Read the supplied [Material Safety Data Sheet](#) before handling CyTRAK Orange™
  2. Since no washing step is required, CyTRAK Orange™ will usually be the final staining procedure, after any cell treatment or labelling, prior to analysis.
  3. Prepare cells for staining with CyTRAK Orange™. Resuspend cells in appropriate buffer such as PBS at a concentration of  $\leq 4 \times 10^5$  / ml in a test tube. For adherent cells estimate the number of cells based on confluence level or tissue section dimensions.
  4. Add CyTRAK Orange™ directly as supplied following the 10  $\mu$ M or 20  $\mu$ M pipetting volumes in **table 1** below. This will be as an overlay for adherent cells / tissue sections, added to the chamber/well liquid directly or in fresh medium following a wash step.
  5. Gently mix and then incubate for 20-60 minutes at room temperature. **NOTE:** Protect from the light during this incubation period if other (immuno-) fluorescent stains have been applied to the cells, prior to the CyTRAK Orange™ labelling, and which may otherwise suffer photo-bleaching. CyTRAK Orange™ staining is accelerated at 37°C and maybe reduced to 10-15 min. CYTRAK Orange™ stains intact, live, fixed, permeabilized and dead cells.
  6. Cells can be analysed directly without further treatment or washing. **NOTE:** To maintain the capacity in the assay for cell compartment discrimination we do not recommend a further washing protocol. If washing is necessary then the cytoplasmic signal will be removed, however the nuclear signal is well maintained.
-

**Considerations:**

It is important to consider the combinations of fluorochromes and filters for the experiment:

**EXCITATION:** CyTRAK Orange™ may be excited by a wavelength range from 488 nm up to 540 nm (Exλ<sub>max</sub> 520 nm) (see [excitation spectra](#) for CyTRAK Orange™).

- 488 nm excitation offers the most simple and optimal wavelength additionally it is available on most imaging instruments.
- **NOTE:** The key benefit of CyTRAK Orange™ is that it can be co-excited with eGFP or eYFP, and the emission profile enables it to be robustly separated from either of these fluorescent constructs.

**EMISSION:** this starts at 580 nm (Emλ<sub>max</sub> 615 nm intercalated to dsDNA). See [emission spectra](#) for CyTRAK Orange™

- Suitable filters include 590LP; or a 630/60 nm band-pass or 615/50 nm band-pass for imaging with eGFP or eYFP constructs

*See table 2 below for recommended filter combinations for imaging.*

**Table 1:**

***Ready reckoner for volumes of CyTRAK Orange™ (5mM) required for various cell concentrations:-***

Cell sample preparation:		Volume of CyTRAK Orange™ (as supplied) required for a concentration of:		
No. of cells:	in volume:	5 µM	10 µM	20 µM
1 x 10 <sup>6</sup>	2500 µl	2.5 µl	5 µl	10 µl
4 x 10 <sup>5</sup>	1000 µl	1 µl	2 µl	4 µl
2 x 10 <sup>5</sup>	500 µl	0.5 µl	1 µl	2 µl
1 x 10 <sup>5</sup>	250 µl	0.25 µl	0.5 µl	1 µl
5 x 10 <sup>4</sup>	125 µl	0.13 µl	0.25 µl	0.5 µl

**Table 2:**

*Typical filter combinations for CyTRAK Orange™*

<i>Fluor combination</i>	<i>Camera imaging</i>	<i>Confocal laser scanning microscopy</i>
<b>CyTRAK Orange™ only</b>	<p><i>Propidium iodide filter set:</i> Excitation 535/50 nm; Dichroic Q565LP; Emission 590LP or HQ645/75</p> <p><i>Texas Red® / Cy 3.5 filter set:</i> Excitation 560/40x; Dichroic 595 DCLP; Emission D630/60</p> <p><i>Also other sets:</i></p> <p>Excitation 560/40 nm; Dichroic 595LP; Emission 630/60</p> <p>or Excitation 546/10x BP nm; 580LP; 590 LP</p>	Excitation 488 nm or 534 nm; Emission 580 LP
<b>CyTRAK Orange™ and EGFP</b>	<p>eGFP: Excitation HQ 480/20 nm; Dichroic Q495LP; Emission HQ515/30</p> <p>CyTRAK Orange™: Excitation 560/40 nm; Dichroic 595LP; Emission 630/60</p>	<p>One laser simultaneous excitation at 488 nm; eGFP emission 522/35 nm and CyTRAK Orange™ emission 598/40 nm</p> <p>Two laser sequential excitation at 488nm for eGFP and 534 nm for CyTRAK Orange™; eGFP emission 522/35 nm and CyTRAK Orange™ 598/40 nm</p>