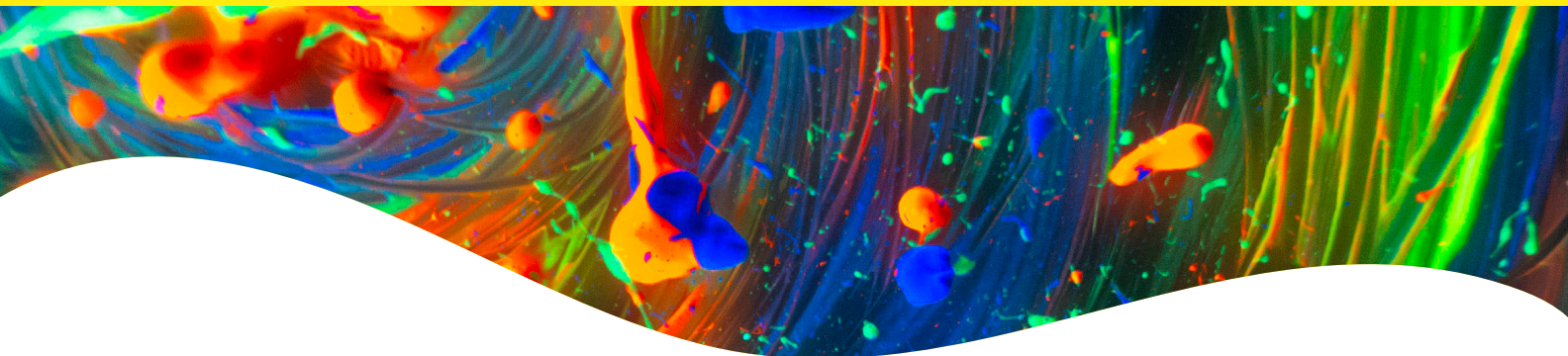


# PhenoVue HypoxiTRAK, Hypoxia Indicator



## Overview

Oxygen molecules support the intracellular energy production and are used by several biochemical processes. Hypoxia, or low oxygen levels, is therefore a significant stress that often impairs aerobic species' ability to survive and is a key component of the pathological conditions such as inflammation, cardiovascular diseases, and cancer. As such, understanding molecular mechanisms underlying hypoxia inducing conditions is crucial. For example, low oxygen level results in the upregulation of Hypoxia inducible factors (HIFs) which in turn regulate the expression of genes involved in cell survival, angiogenesis, glycolysis, and invasion/metastasis.

PhenoVue™ HypoxiTRAK™ is a novel, far-red fluorescing dye that is activated by bio-reduction and is sensitive to the degree of biologically relevant levels of hypoxia. The fluorescence signal accumulated is equivalent to the hypoxic experience of each cell, and PhenoVue HypoxiTRAK allows direct time-lapse monitoring of the accumulated hypoxic experience. While being non-toxic to normoxic cells, exposure to continuous or periodic hypoxia will lead to the proportionate intracellular accumulation of PhenoVue HypoxiTRAK in affected cells, ultimately inducing cell arrest and enabling a direct read-out of a hypoxic fraction. The hypoxia sensing range for HypoxiTRAK is relevant to biomarker and hypoxia-targeting drug development.

## Product information

Product name*	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue HypoxiTRAK, hypoxia indicator	CP181	1	500 µL (10 µM - 5 nmol)	Liquid	Ambient

\*HypoxiTRAK™ is a trademark of BioStatus Limited.

## Storage and stability

- **Do not freeze.**
- Store at 2-8 °C, protected from light.
- The stability of these products is guaranteed until the expiration date indicated on the vial, when stored as recommended and protected from light.
- Allow the reagents to warm up to room temperature for 15 mins before opening the vials, and aliquot.
- Aliquoted reagents must be stored at 2-8 °C.

## Equivalent number of microplates

Product name	When used at recommended concentration	96-well microplate (100 $\mu$ L - 300 $\mu$ L per well)	384-well microplate (25 $\mu$ L - 90 $\mu$ L per well)	1536-well microplate (4 $\mu$ L - 12 $\mu$ L per well)
PhenoVue HypoxiTRAK, hypoxia indicator	50 nM	Approx. 3 to 10	Approx. 3 to 10	Approx. 5 to 16

View our full range of high-quality imaging microplates at [Revvity.com](http://Revvity.com)

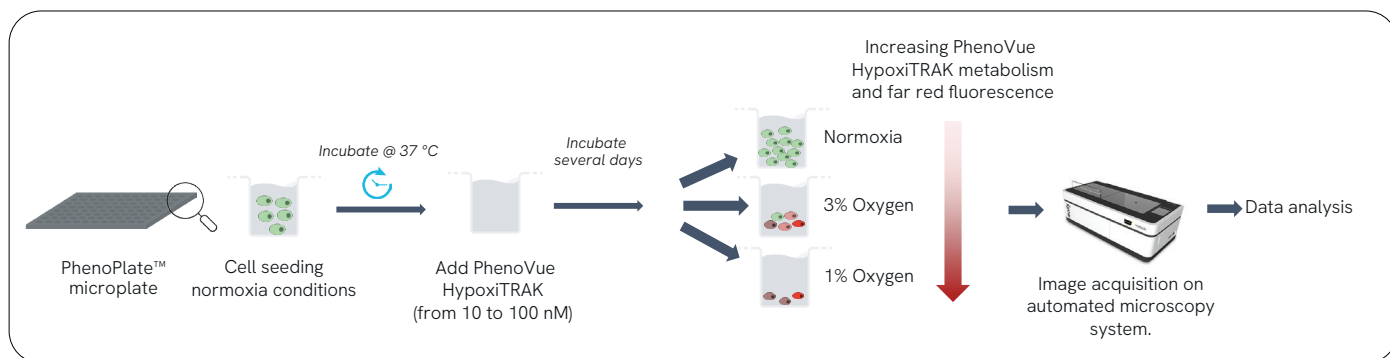
## Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Filter set
PhenoVue HypoxiTRAK, hypoxia indicator	646	697	Cy5

## Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue HypoxiTRAK, hypoxia indicator	Yes	Yes	No

## Experimental workflow



## Protocols

### Cell culture

Seed cells in imaging black wall, clear bottom microplates (or any other convenient cell culture vessels). Incubate in the appropriate cell culture conditions, usually 37 °C, 5% CO<sub>2</sub> until 50-70% confluency.

### Staining

To report hypoxia, PhenoVue HypoxiTRAK is added to the culture medium at the appropriate time in the protocol. It does not need to be replaced for analysis of the cells. PhenoVue HypoxiTRAK can be diluted in culture media (e.g. RPMI 1640) and physiological buffers (e.g. PBS, Hanks's, etc.).

1. Prepare cell cultures with treatments, if any, to be subjected to hypoxia (t = 0): monolayer in early growth phase or suspension culture at approximately 2 x 10<sup>5</sup> cells/mL. If required, harvest a parallel culture at the start of the experiment to establish culture density.
2. Add PhenoVue HypoxiTRAK directly to cultures: initially use a final concentration range of 10, 30 or 100 nM to test for dynamic range with respect to the prevailing culture density and extent of hypoxia.
3. Incubate cultures under the selected hypoxic conditions for chosen periods (t = 2-5 days), during which biologically relevant changes to cell behavior are under study.
4. Acquire images using an imaging system such as the Opera Phenix® Plus high-content screening system.

### Tips

PhenoVue HypoxiTRAK-metabolite inhibits cell division - marking and preserving cells that have experienced hypoxic conditions.

### Safety information

Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Applications

- High-content analysis/high-content screening
- Imaging microscopy

## Validation data

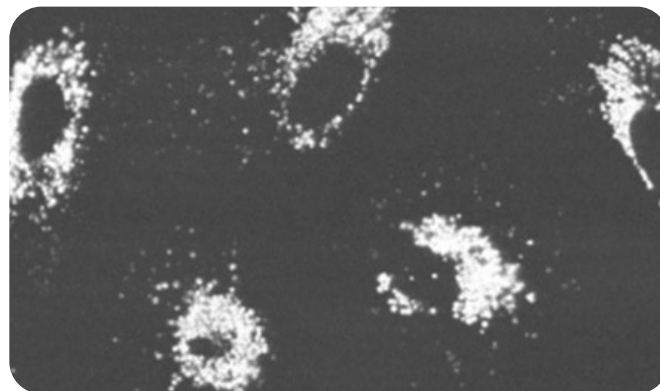


Figure 1: Cellular accumulation of PhenoVue HypoxiTRAK-metabolite in A549 cells exposed to 100 nM HypoxiTRAK for 4 days under 1% oxygen.

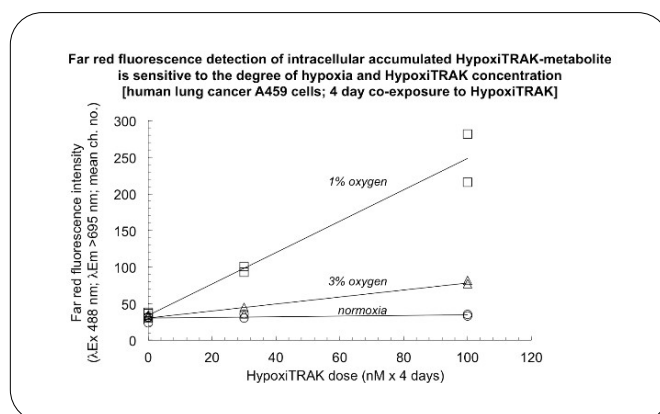


Figure 2: Analysing cellular experience of hypoxia using PhenoVue HypoxiTRAK. Analysis of degree of hypoxic experience over 4 days in cells co-exposed to PhenoVue HypoxiTRAK. PhenoVue HypoxiTRAK added directly to monolayer cell cultures prior to incubation at different levels of oxygenation. Cells harvested using conventional method and analysed with imaging system using Cy5 filter set.

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