HypoxiTRAK™ TECHNICAL DATA SHEET

PRODUCT: HypoxiTRAK™ **PRESENTATION:** aqueous solution.
PRODUCT NUMBERS: HT10500 **STORAGE**: store at 2-8 ºC. Do not freeze.

DESCRIPTION:
HypoxiTRAK™ is a novel, far-red fluorescing dye that reveals the hypoxic experience of individual cells. HypoxiTRAK™ is non-toxic to normoxic cells. HypoxiTRAK™ can be used in flow cytometry and imaging protocols and is spectrally compatible with common visible range fluors including FITC and R-PE. HypoxiTRAK™ enables entirely new assay approaches to functional hypoxia.

APPLICATIONS:
Reporting hypoxia experience in cell cultures, spheroids and tissues:
- Flow Cytometry – dynamic live cell hypoxia study
- Fluorescence Microscopy – dynamic live cell hypoxia study; immuno-fluorescence

BACKGROUND INFORMATION:
The role of hypoxia in cancer and stem cells is an expanding area of research. Low oxygen stress can change biological behaviour and pharmaco-dynamic responses due to hypoxia-induced activation of cell protective and proliferation mechanisms. HypoxiTRAK™ is a novel molecular probe for use in flow cytometry and imaging to report (i) the degree of hypoxic experience of cells and (ii) the functionally hypoxic fraction of cells.

HypoxiTRAK™ is designed to activate by bio-reduction at biologically relevant levels of hypoxia, given that *in vivo* tumour cells can occupy hypoxic niches with lower median oxygen levels (~1 % oxygen; pO_2 7.5 mmHg) compared to normal tissues (~5.5% oxygen; pO_2 42 mmHg) while tumour cores may maintain a less than 0.1% oxygen (pO_2 1 mm Hg) environment. The hypoxia sensing range for HypoxiTRAK™ is relevant to biomarker and hypoxia-targeting drug development.

The bioactive metabolite retains the parent fluorophore. This accumulates in cells, proportional to the extent of hypoxia experienced, to provide a persistent far-red fluorescent signature and induces cell arrest, thereby marking and 'freezing' the hypoxic cells, enabling a direct read-out of a hypoxic cell fraction.

HypoxiTRAK™ allows assessment of the degree of hypoxia by simple assays for growth arrest or apoptosis. HypoxiTRAK™ bio-activation, resulting in prolonged cell cycle arrest, can be detected by increased side scatter by flow cytometry. As no fixation is required, HypoxiTRAK™ can provide a convenient negative selection for cells not experiencing significant hypoxia within heterogeneous populations, including 3D culture. HypoxiTRAK™ shares spectral characteristics with the related cell-permeant DNA dye DRAQ5™, which labels all nucleated cells, and can be used as a convenient standard to mark all such cells within a sample in parallel with analysis for hypoxic fraction.

HypoxiTRAK™ is typically present during the period of hypoxia under investigation. The physicochemical properties of HypoxiTRAK™ facilitate permeation throughout populations of cells in 3-D culture and tissues thereby enhance its entry into hypoxic niches and reducing retention upon cell isolation, providing an excellent signal:noise. HypoxiTRAK™ is ideal for studies over several days. HypoxiTRAK™ read-out is direct, obviating cell processing. HypoxiTRAK™ shows low perturbation in the absence of bio-activation while the unique far-red fluorescence signature offers compatibility with other end-point assays for hypoxia that use fluorescence signatures.

Oxygenation levels vary with regard to extent and duration; HypoxiTRAK™ provides a retrospective/historical reporting of the integrated hypoxic experience of a cell – it is a sensor of the hypoxia experienced by each cell within an *in vitro* cell population.

SPECTRAL CHARACTERISTICS:
Exλ_{max} 600/646 nm Emλ_{max} 697 nm

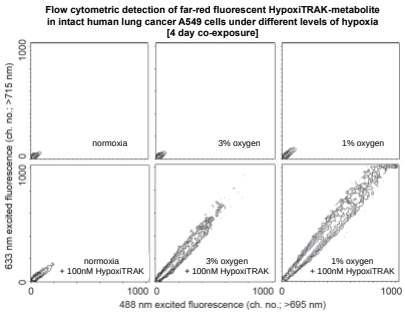

Fig. 1 Flow cytometry of HypoxiTRAK™-metabolite, blue & red excitation; intact A549 cells under hypoxia (3%, 1%), with & without HypoxiTRAK™ [4 day co-exposure]

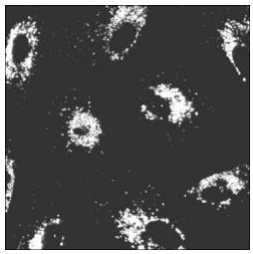

Fig. 2 HypoxiTRAK™-metabolite accumulates in cytoplasm of A549 cells (ex. 633 nm; em. 680/20 nm) exposed to 100 nM HypoxiTRAK™ for 4 days 1% O_2

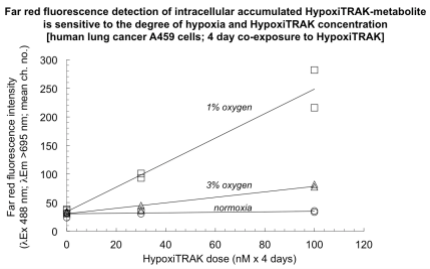

Fig.3 Intracellular accumulation of HypoxiTRAK™-metabolite is sensitive to degree of hypoxia and [HypoxiTRAK] (A549 cells, conditions as fig. 2)

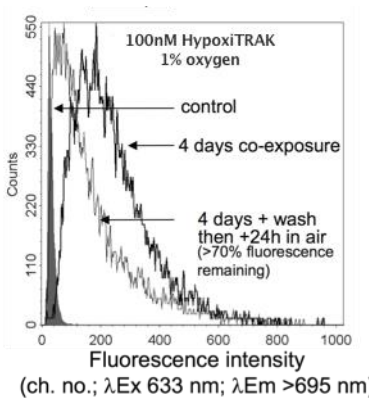

Fig. 4 HypoxiTRAK™-metabolite persistence: intact A549 cells retain >70% after washing and 24h incubation in HypoxiTRAK™-free medium (ex. 633nm; em. >695nm)

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

MATERIALS OFTEN REQUIRED BUT NOT SUPPLIED:
Phosphate-Buffered Saline (PBS, without azide), culture medium, Paraformaldehyde, Antibodies, Plastic-ware.

****NOTE:** retained HypoxiTRAK™-metabolite inhibits cell division - marking and preserving cells that have experienced hypoxic conditions.

DETECTING HypoxiTRAK™ SIGNALS: (see Fig. 1)
Flow cytometry: HypoxiTRAK-metabolite™ can be excited by 488 nm wavelength light. Detect this in a 660/16 nm channel. Exposure of the cells to the unconverted HypoxiTRAK™ is proven by excitation at 635 nm and detection in the 670LP channel.
Microscopy / HCS Imaging Platform: HypoxiTRAK™ is optimally excited using yellow / red wavelengths. It is detected with far-red filters above 660 nm.

EXAMPLE PROTOCOL
To report hypoxia, 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.

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 10^5 cells/ml. If required harvest a parallel culture at the start of the experiment to established culture density.

2. Add HypoxiTRAK™ directly to cultures: initially use a final concentration range of 10, 30 or 100 nM HypoxiTRAK™ 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 behaviour are under study.

4. Analysis:
   For adherent cultures – wells can be imaged as required in real-time, or cells harvested by an appropriate method, washed to remove debris and re-suspended in cold medium prior to conventional flow cytometry.
   For suspension cultures - samples can be used directly without any processing and analysed by flow cytometry.

   Samples (e.g. suspension cells, adherent cells, cryo-preserved sections) requiring further staining for immunofluorescence should be fixed with 4% formaldehyde for 5 minutes. Care should be taken to limit PBS washes to a minimum to avoid wash-out of HypoxiTRAK™-metabolite, prior to immune-staining.

   Cell enumeration will reveal reciprocity between cell proliferation capacity, viability and HypoxiTRAK™ bioactivation.

WHAT YOU SHOULD EXPECT TO SEE
In cells that have experienced functional hypoxia, HypoxiTRAK™ accumulates in the cytoplasm (see fig. 2) and can be detected by fluorescence microscopy or by flow cytometry (figs. 1, 3, and 4). Flow cytometric data for each time point should be displayed as Cumulative Distribution Function (CDF) compared to the control(s). As a far-red fluorescing dye HypoxiTRAK™ signals are spectrally separated from the majority of visible-range chromophores.

For Research Use Only.

BioStatus products are the subject of several international patents.
HypoxiTRAK™ is a trademark of BioStatus Limited.

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