

# BioTracker™ 520 Green Hypoxia Dye

Live Cell Dye

Cat. # SCT033

FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.  
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

pack size: 5x25µg

Store at -20°C



## Data Sheet

page 1 of 2

### Background

Hypoxia is defined as the reduction or lack of oxygen in organs, tissues, or cells. Hypoxia in living tissue closely relates to a wide array of pathologies, including cancer and ischemia. Existing hypoxia detection probes, such as pimonidazole, require cell fixation followed by immunostaining.

The BioTracker™ 520 Green Hypoxia Dye is a fluorescent imaging probe for the detection of hypoxia in living cells. This probe is as sensitive as pimonidazole and can be used for live cell fluorescence imaging and flow cytometry applications.

### Storage

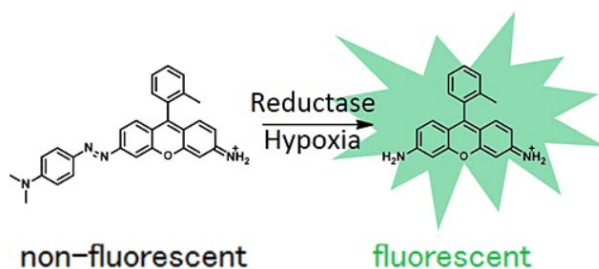
Store BioTracker 520 Green Hypoxia Dye at -20°C, desiccate and protect from light

*Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.*

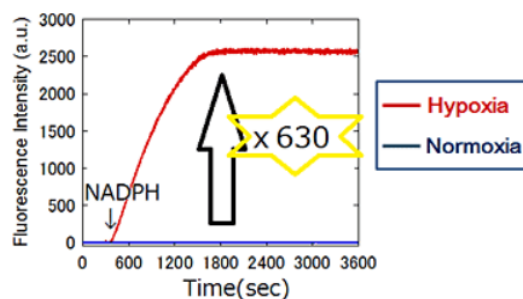
### Spectral Properties

Absorbance: 498nm

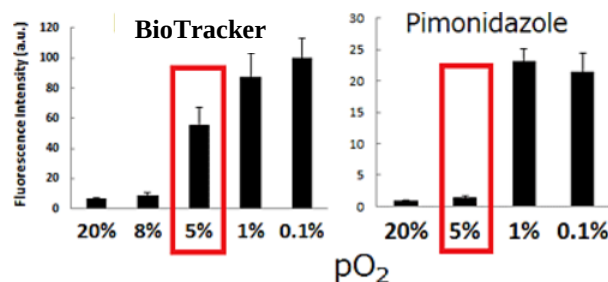
Emission: 520nm



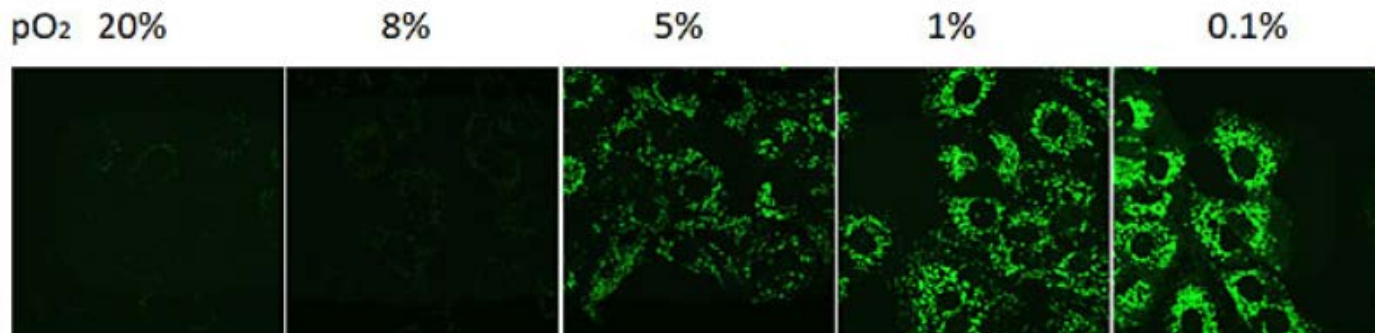
**Figure 1.** BioTracker 520 Green Hypoxia Dye mechanism



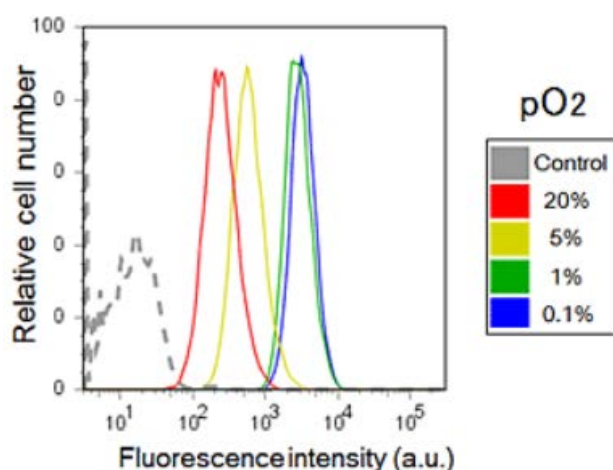
**Figure 2.** NADPH was introduced to 5 µM BioTracker 520 Green Hypoxia Dye in the existence of rat liver microsomes, under hypoxia or normoxia. Fluorescence was over 100 times brighter under hypoxic conditions.



**Figure 3.** Fluorescent intensity in A549 cell under hypoxia. A549 cells were observed after staining with BioTracker 520 Green Hypoxia Dye or pimonidazole under the various concentrations of oxygen. While pimonidazole responded to the concentration of oxygen under the 1%, BioTracker responded to the oxygen concentration of about 5%.



**Figure 4.** Fluorescent imaging of live A549 cell under the various concentration of oxygen using BioTracker 520 Green Hypoxia Dye.



**Figure 5.** A549 cells were analyzed by flow cytometry after incubation for 6 hours under the various oxygen concentrations and stained by 1  $\mu$ M BioTracker 520 Green Hypoxia Dye. Fluorescent intensity increased as the oxygen concentration decreased, indicating that the dye is a viable probe for hypoxia detection using flow cytometry.

## Protocol

### Reagent Preparation

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Add 43  $\mu$ L of DMSO to one vial of dye to achieve a 1 mM stock solution.
3. Dilute the stock solution with DMEM to achieve a 5  $\mu$ M cell stain solution.
4. Remove cell culture media and rinse twice with PBS. Add stain solution to the dish and incubate for 1 hour.
5. Remove stain, wash with PBS and replace with DMEM and observe the cells using a fluorescence microscope after 3 hours of incubation time.

BioTracker™ is a trademark of Merck KGaA

antibodies Multiplex products biotools cell culture enzymes kits proteins/peptides siRNA/cDNA products

Please visit [www.millipore.com](http://www.millipore.com) for additional product information, test data and references

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500

Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502

**FOR RESEARCH USE ONLY.** Not for use in diagnostic procedures. Not for human or animal consumption. Purchase of this Product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

EMD Millipore®, the M mark, Upstate®, Chemicon®, Linco® and all other registered trademarks, unless specifically identified above in the text as belonging to a third party, are owned by Merck KGaA, Darmstadt, Germany. Copyright ©2008-2018 Merck KGaA, Darmstadt, Germany. All rights reserved.



We Buy 100% Certified Renewable Energy