molecular probes
life technologies

Image-iT® Hypoxia Reagent

Catalog no. H10498

Table 1. Contents and storage

Material	Amount	Storage	Stability
Image-iT® Hypoxia Reagent	1 mg	• ≤–20°C • Desiccate • Protect from light	When stored as directed, this product is stable for at least 6 months.
MW: 711.87 **Approximate Ex/Em maxima:** 490/610 nm (see Figure 1, page 2).			

Introduction

Image-iT® Hypoxia Reagent is a live-cell permeable compound which increases fluorescence in environments with low oxygen concentrations. Unlike pimonidazole adducts, which respond to very low oxygen levels, Image-iT® Hypoxia reagent is fluorogenic when atmospheric oxygen levels are less then 5% (Figure 2, page 2). The Image-iT® Hypoxia Reagent shows real time response to oxygen levels in live cells by increasing signal with reduced oxygen. These properties make this reagent an ideal tool for detecting hypoxic conditions around tumor cells, 3D cultures, spheroids, neurons, and other live samples. Image-iT® Hypoxia reagent can be used to detect tumors in small animals, and its fluorogenic properties correspond with increased Hif 1α expression and translocation under hypoxic environments.[1]

Before you begin

Prepare stock solutions

Image-iT® Hypoxia Reagent is provided as a lyophilized powder. To make 1 mM stock solution, dissolve the lyophilized powder in 1.40 mL of DMSO. This stock solution can be stored at ≤–20°C for 6 months, or 2°C to 8°C for up to one week. For best results, mix well when preparing the stock solution and avoid the freeze-thaw cycles.

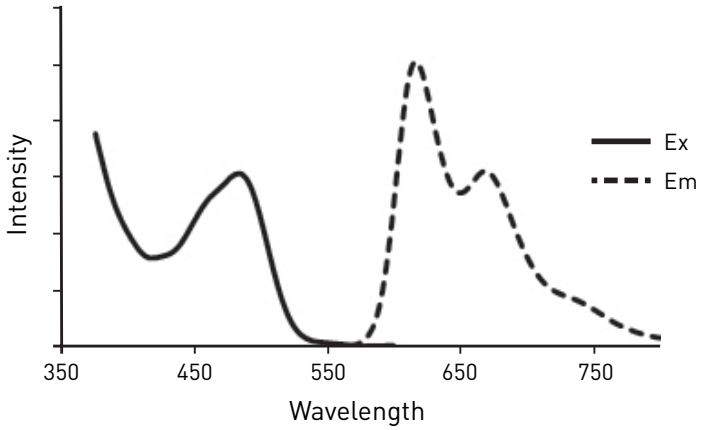
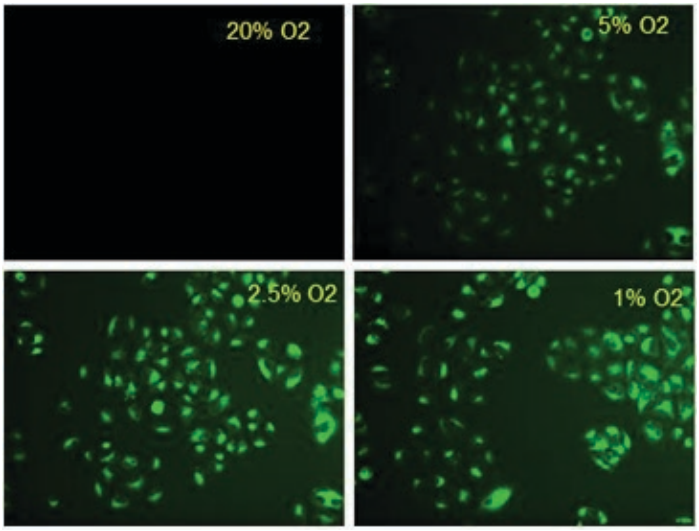


Figure 1. Typical absorption and emission spectra of Image-iT® Hypoxia Reagent



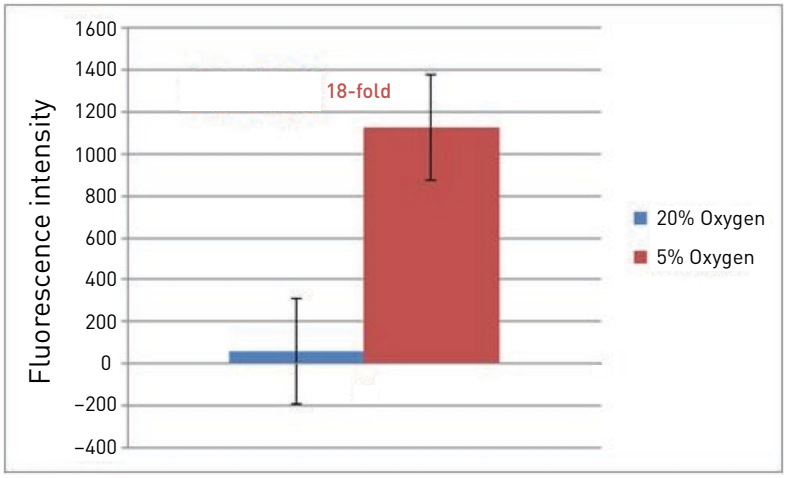


Figure 2. A549 cells were grown on a glass-bottom 30-mm dish at a density of 1 × 10^5 cells/dish and left overnight under normoxic conditions in a CO_2 incubator. A 5-µM solution of Image-iT® Hypoxia reagent was added to the cells in complete growth medium. The cells were then incubated on an EVOS® Onstage Incubator at varying O_2 concentrations (20%, 5%, 2.5% and 1%) for 1 hour and then imaged on the EVOS® FL Auto fluorescence microscope using a special filter with excitation of 488 nm and emission of 610 nm.

Experiment guidelines

1. Plate cells at the recommended density on a glass bottom dish and incubate them overnight at 37°C overnight in a CO_2 incubator. For better results, higher cellular density is recommended.

2. Prepare a 1 mM stock solution of the Image-iT® Hypoxia Reagent in DMSO. Add the reagent to the cells at a final concentration of 5–10 µM and incubate for 15 to 30 minutes.

3. Place the cells in a hypoxia chamber set to the desired oxygen concentration and attached to a fluorescence microscope, and incubate the cells .

4. Image the cells on the fluorescence microscope with excitation/emission of 490/610 nm.

   Note: You can use the standard TRITC filter set for imaging; however, we recommend using FITC excitation and Texas Red® emission filters for best results.

References

1. J Immunol Methods 313, 199 (2006).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
H10498	Image-iT® Hypoxia Reagent	1 mg
Related Products		
A14291DJ	Live Cell Imaging Solution	500 mL
A1896701	FluoroBrite™ DMEM	500 mL
D12345	DMSO, anhydrous	10 × 3 mL

Purchaser notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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