Image-iT™ Hypoxia Reagents

Catalog No. H10498, I14833, I14834

Pub. No. MAN0013497 **Rev.** C.0

Product information

Image-iT™ Red Hypoxia Reagent and Image-iT™ Green Hypoxia Reagent are live-cell permeable compounds which increase fluorescence in environments with low oxygen concentrations. Unlike pimonidazole adducts, which only respond to oxygen levels lower than 1%, Image-iT™ Red Hypoxia Reagent and Image-iT™ Green Hypoxia Reagent are fluorogenic when atmospheric oxygen levels are lower than 5%, and their fluorogenic response increases as the oxygen levels decrease in the environment (Figures 2–3).

The Image-iT™ Red Hypoxia Reagent responds to reduced oxygen levels in live cells by increasing signal in real-time, and this increase is reversible as oxygen levels improve. The Image-iT™ Green Hypoxia Reagent is an end-point assay reagent, whose signal increases with reduced oxygen levels, but is not reversible. This signal is formaldehyde-fixable, but it doesn't survive detergent permeabilization. These properties make the Image-iT™ Hypoxia Reagents ideal tools for detecting hypoxic conditions around tumor cells, 3D cultures, spheroids, neurons, and other live samples (Figure 5).

The Image-iT™ Hypoxia reagents can be detected with all common instruments capable of detecting a fluorescent signal, such as wide-field microscopes, confocal microscopes, fluorescent plate readers, and high-throughput screening (HTS) and high-content analysis (HCA/HCS) instruments (Figures 2–5). Image-iT™ Red 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]

Table 1. Contents and storage

Product	Catalog No.	Amount	Storage*
Image-iT™ Red Hypoxia Reagent	H10498	1 vial	• Store at ≤–20°C upon receipt. • Desiccate. • Protect from light.
Image-iT™ Green Hypoxia Reagent	I14833	5 vials	
	I14834	1 vial	
* When stored as directed, the product is stable for at least 6 months from the date receipt.			

Table 2. Characteristics of Image-iT™ Hypoxia Reagents

Product	Approximate Ex/Em maxima*	Reversible signal	Endpoint detection	Fixable (Methanol-free 4% PFA)
Image-iT™ Red Hypoxia Reagent	490/610 nm	Yes	No	No
Image-iT™ Green Hypoxia Reagent	488/520 nm	No	Yes	Yes
* See Figure 1, page 2.				

Figure 1. Typical absorption and emission spectra of **(A)** Image-iT™ Red Hypoxia Reagent and **(B)** Image-iT™ Green Hypoxia Reagent.

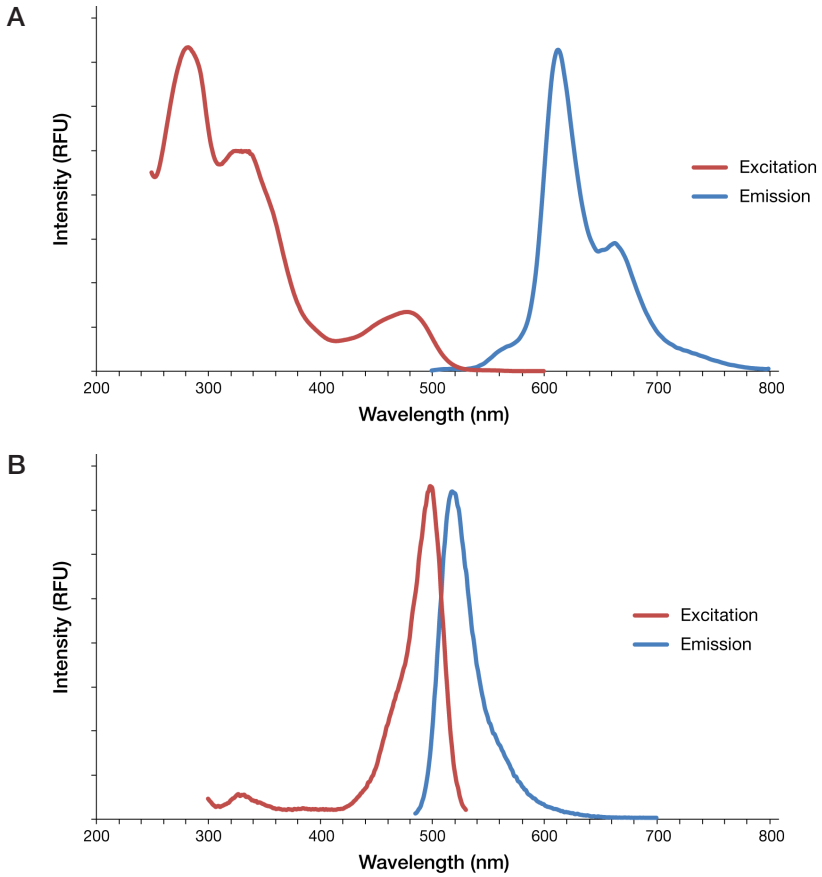


Figure 2. A549 cells were grown on a glass-bottom 30-mm dish at a density of 1 × 10^5 cells/dish and left overnight under normoxic conditions in a CO_2 incubator. A 5-µM solution of Image-iT™ Red Hypoxia Reagent was added to the cells in complete growth medium. The cells were then incubated in an EVOS™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

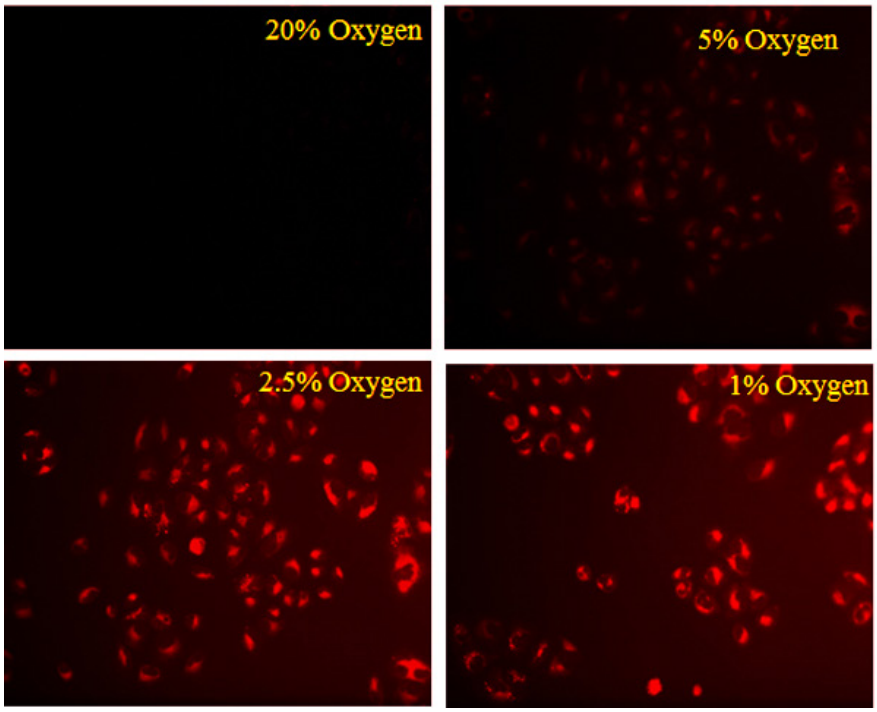


Figure 3. A549 cells were plated on MatTek dishes at a density of 1 × 10^5 cells/dish and incubated overnight in a CO_2 incubator at 37°C. The next day, existing medium from cells was removed and replaced with fresh growth medium containing the Image-iT™ Green Hypoxia Reagent at a final concentration of 5 µM. The cells were incubated at 20% O_2, 5% O_2, 2.5% O_2, or 1% O_2 for 3 hours. The cells were then washed twice with Live Cell Imaging Solution (LCIS, Cat. No. A14291DJ) and imaged on a Zeiss™ 710 confocal microscope.

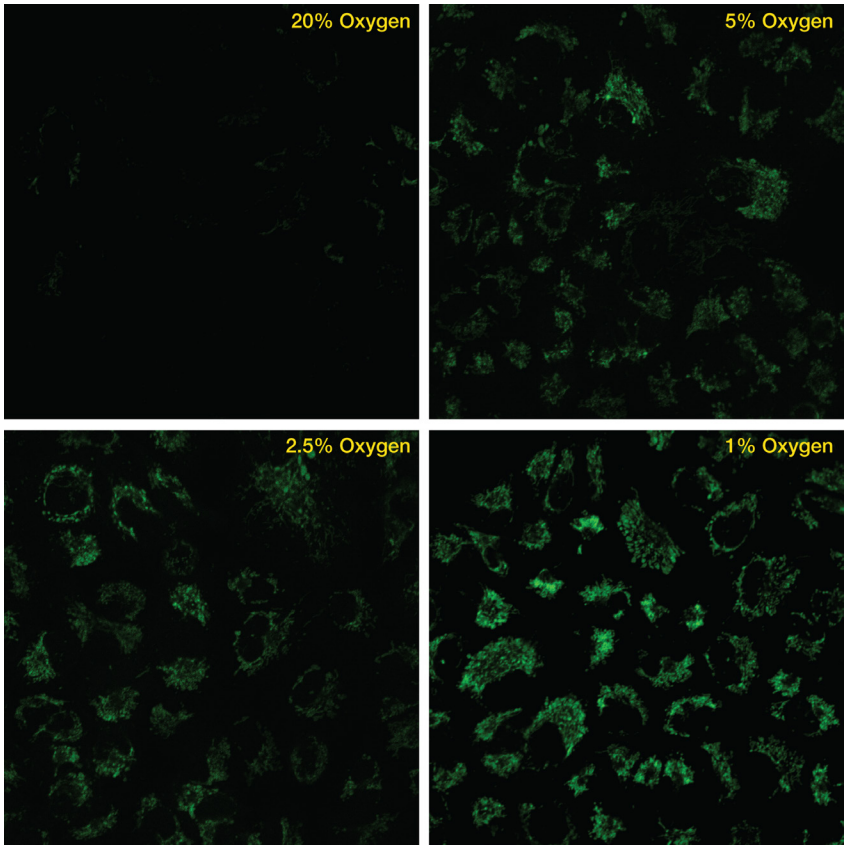


Figure 4. Analyzing the cells under hypoxic conditions with a fluorescent plate reader (left) or with a High Content Analysis instrument (right). A549/HeLa/U2OS cells were plated on a Greiner 96-well plate at a density of 7 × 10^3 cells/well and incubated overnight in a CO_2 incubator at 37°C. The next day, existing medium from cells was removed and replaced with fresh growth medium containing the Image-iT™ Green Hypoxia Reagent at a final concentration of 5 µM. The cells were incubated at 20% O_2 or 1% O_2 for 5 hours. The cells were then washed twice with Live Cell Imaging Solution (LCIS, Cat. No. A14291DJ) and stained with Hoechst 33342 (2 µM) Reagent. Cells were then analyzed either on the Thermo Scientific™ Varioskan™ LUX multimode microplate reader (left) or Thermo Scientific™ Cell Insight CX5 HCS instrument (right).

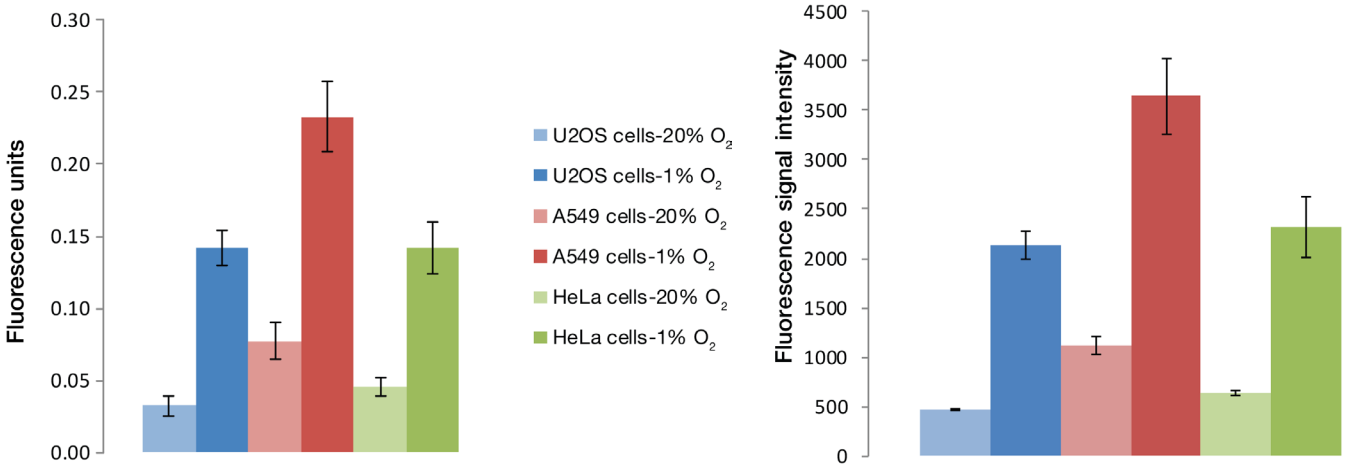
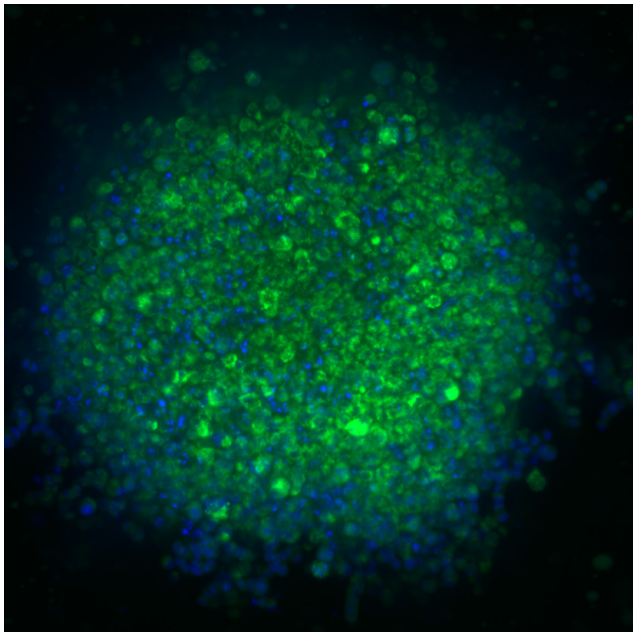


Figure 5. Hypoxic core staining of A549 spheroid with Image-iT™ Green Hypoxia Reagent. A549 cells were plated at a density of 5000 cells/well in a 96-well Corning™ spheroid plate and incubated for 48 hours under normoxic conditions. The spheroids were then stained with 5 µM Image-iT™ Hypoxia Green probe (green) and Hoechst 33342 dye (blue). The plate was automatically imaged with a 10X objective using confocal mode on a Thermo Fisher Scientific™ Cell Insight CX7LZR HCS instrument. The image is from a maximum intensity projection of 20 optical Z slices of 10 microns each.



Before you begin

Prepare stock solutions

- **Image-iT™ Red Hypoxia Reagent:** Image-iT™ Red Hypoxia Reagent is provided as a lyophilized powder. To prepare a 1 mM stock solution, dissolve the lyophilized powder in 1.40 mL of DMSO and mix well. This stock solution can be stored at ≤–20°C for 6 months, or at 2°C to 8°C for up to one week. For best results, avoid freeze-thaw cycles.
- **Image-iT™ Green Hypoxia Reagent:** Image-iT™ Green Hypoxia Reagent is provided as a lyophilized powder. To prepare a 5 mM stock solution, dissolve the lyophilized powder in 10 µL of DMSO and mix well. This stock solution can be stored at ≤–20°C for up to a month.

Experiment guidelines

1. **Monolayer cells:** Plate the cells at the recommended density on a glass bottom dish or a 96-well plate and incubate them overnight in a CO_2 incubator at 37°C, then proceed to step 2. For better results, higher cellular density is recommended.

   Live tissue and 3D cultures (Spheroids): Maintain the live tissue or grow 3D cultures as recommended by the vendor. Move the tissue or culture to a glass bottom dish with fresh medium, then proceed to step 2.

2. Add the Image-iT™ Hypoxia Reagent stock solution to the cells at a final concentration of 1–10 µM in the appropriate live cell medium, then incubate at 37°C for 30 minutes for monolayer cultures or 1 hour for 3D spheroids.

   Note: The recommended concentrations are based on experiments performed with A549, HeLa, and MMM cells. Optimize the dye concentrations for the cell line you are using. For Jurkat cells detected with a flow cytometer, we have observed that 0.5–1.0 µM concentration worked the best.

3. Exchange the media with fresh growth medium. Place the cells in a cell culture incubator at 20% O_2 or in a hypoxia chamber/incubator set to the desired oxygen concentration, then incubate for 2–4 hours. Live tissue and 3D cultures might require longer incubation, depending on the thickness of the tissue.

   Note: Incubation in the hypoxia chamber is optional for 3D cultures or spheroids with a natural hypoxic core.

4. Image the cells using a fluorescence microscope with excitation/emission as given in Table 2 (page 1).

   - For Image-iT™ Red Hypoxia Reagent, we recommend using FITC excitation and Texas Red™ emission filters for best results. TRITC standard excitation/emission filter set can also be used.
   - For Image-iT™ Green Hypoxia Reagent, we recommend using standard FITC/GFP excitation/emission filter set.

   Note: If needed, Image-iT™ Green Hypoxia Reagent can be fixed with methanol-free 4% formaldehyde. Image within 24 hours after fixation. There may be some decrease in signal intensity after fixation; adjust the microscope setting to get the optimal signal. Detergent permeabilization is not recommended.

References

1. Cancer Res 70 (11), 4490–4498 (2010).

Ordering information

Cat. No.	Product name	Unit size
H10498	Image-iT™ Red Hypoxia Reagent	1 vial
I14833	Image-iT™ Green Hypoxia Reagent	5 vials
I14834	Image-iT™ Green Hypoxia Reagent	1 vial
Related products		
A14291DJ	Live Cell Imaging Solution	500 mL
A1896701	FluoroBrite™ DMEM	500 mL
D12345	DMSO, Anhydrous	10 × 3 mL

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history: Pub. No. MAN0013497

Revision	Date	Description
C.0	01 February 2018	Correct the amount of Image-iT Hypoxia Reagent supplied.
B.0	05 October 2017	Add information about Image-iT Green Hypoxia Reagent, update figures, rebrand.
A.0	05 February 2015	New user guide

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