Intracellular Detection of Hypoxia in Live Cells

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Overview
Hypoxia is an important phenomenon in many physiological processes and involved in many human diseases including cancer. Information can lead to significant hypoxia in tissues. The study of hypoxia has been complicated with the lack of proper instrumentation to induce hypoxia in cells and image cells under hypoxic conditions. Here, we describe a live-cell method to conveniently measure hypoxia using a new Image-IT™ Hypoxia Probe and a specialized microscope incubator which can control oxygen concentrations down to 1%. The Image-IT™ Hypoxia Probe is an oxygen sensing fluorescent probe, it is quenched with increasing oxygen concentrations, and it has excitation and emission peaks of 483 and 616 nm respectively. The probe is sensitive to varying concentrations of oxygen and can detect as low as 1% O₂ concentrations in cells. Imaging of cells with the Image-IT™ Hypoxia Probe in the incubator prevents re-oxygenation of cells and gives more precise measurement of hypoxia in cells, allowing for reversible and dynamic measurements of hypoxia in cells. Using this system, we measured hypoxia in several cell lines including A549, HeLa and U-2 OS. The Image-IT™ Hypoxia Probe is also very good at detecting hypoxia in 3D tumor spheroids generated using different methods. The new hypoxia probe gives good signal to noise with more than 3-fold changes at 5% O₂ levels with robust statistics. The Image-IT™ Hypoxia Probe provides a good system for precise, robust and reproducible measurements of hypoxia in cells.

Introduction
Image-IT™ Hypoxia Reagent is a fluorogenic compound that becomes fluorescent in environments with low oxygen concentrations, and it is live cell permeable. These properties make it a highly useful tool for detecting cells and imaging in hypoxic conditions. Image-IT™ Hypoxia Reagent is a very sensitive oxygen detector. Unlike pimonidazole adducts that respond only to very low oxygen levels, Image-IT™ Hypoxia Reagent begins to fluoresce when atmospheric oxygen levels are less than 5%. It responds quickly to such environments, and the fluorogenic response reverses if oxygen concentrations improve. These properties make Image-IT™ Hypoxia Reagent an ideal tool for detecting hypoxia conditions around tumors, 3D cultures, spheroids, neurons, etc. It can be used to detect tumors in small animals, and its fluorogenic properties have been shown to correspond with increased HIF1α expression and translocation in hypoxic environments (1).

Features of Image-IT™ Hypoxia Reagent include:

- Measures hypoxia in live cells by fluorescing in low oxygen environments
- Real-time oxygen detector, with reversible fluorogenic response
- Easy to use—just add to cell culture media and image

Figure 1: Excitation/emission spectra of Image-IT™ Hypoxia Reagent

The peak excitation and emission of Image-IT™ Hypoxia Reagent is 490 nm and 630 nm respectively.

Figure 2: Protocol for Live cell staining with Image-IT™ Hypoxia Reagent

Figure 3: A549 cells were grown on Nanoculture™ 38 mm glass bottom dishes in complete medium. The cells were incubated in FluoroBrite® DMEM with 5 µM Image-IT™ Hypoxia Probe at different levels of oxygen (20%, 5%, 2.5% and 1%) for 1 hr on an EVOS®-FL Auto Imaging system. The images were taken after 1 hr of incubation at each oxygen level. The hypoxia signal can be detected at 5% O₂ levels.

Figure 4: A549 HeLa or U2OS cells were grown on Nanoculture™ 38 mm glass bottom dishes in complete medium. The cells were incubated in FluoroBrite® DMEM with 5 µM Image-IT™ Hypoxia Probe at 2.5% oxygen (hypoxic) for 1 hr on an EVOS®-FL Auto Imaging system. The images were taken on an EVOS®-FL Auto Imaging system.

Figure 5: Hypoxia Imaging in 3D Spheroids with Image-IT™ Hypoxia Reagent

References

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