Intracellular Detection of Hypoxia in Live Cells

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Overview

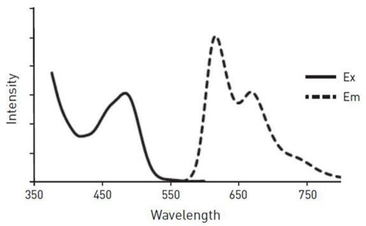
Hypoxia is an important phenomenon in many physiological processes and involved in many human diseases including cancer. Inflammation can lead to significant hypoxia in tissues. The study of hypoxia has been complicated with the lack of proper instrumentation to induce hypoxia in cells and image cells under hypoxic conditions. Here, we describe a live cell-based 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, is quenched with increasing oxygen concentrations, and 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% O_2 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% O_2 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 makes it a highly useful tool for detecting cells and tissue under 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 hypoxic 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 corresponds with increased Hif 1α expression and translocation in hypoxic environments (1).

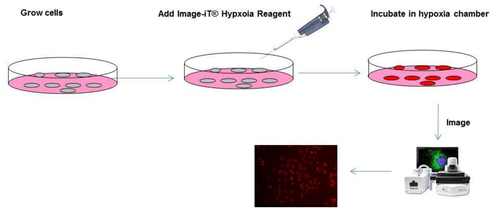
Features of Image-iT™ Hypoxia Reagent include:
- Measures hypoxia in live cells by fluorescing in low oxygen environments
- Real-time oxygen detector, with reversible fluorogenic response
- Easy to use—just add to cell culture media and image

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



The peak excitation and emission of Image-iT™ Hypoxia Reagent is 490 nm and 610 nm respectively

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



Results

Figure 3: Live cell detection of Hypoxia with Image-iT™ Hypoxia Reagent

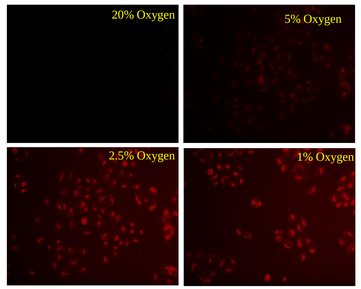


Figure 3: A549 cells were grown on Nunc™ 30 mm glass bottom dishes in complete medium. The cells were incubated in Fluorobrite™ DMEM with 5 µM Image-iT™ Hypoxia Probe at different levels of oxygen (20%, 5%, 2.5% and 1%) for 1 hr on an EVOS® on-stage incubator attached to an EVOS® FLAuto Imaging system. 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: Reversibility of Hypoxia Signal with Image-iT™ Hypoxia Reagent

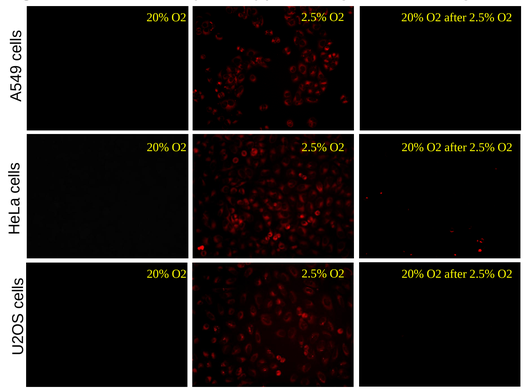
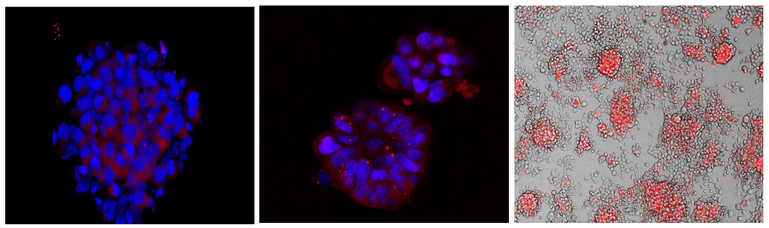


Figure 4: A549, HeLa or U2OS cells were grown on Nunc™ 30 mm glass bottom dishes in complete medium. The cells were incubated in Fluorobrite™ DMEM with 5 µM Image-iT™ Hypoxia Probe at 2.5% oxygen (hypoxic) for 1 hr on an EVOS® on-stage incubator and incubated for another 1 hr after restoring oxygen levels to 20% (normoxic). The images were taken on an EVOS® FLAuto Imaging system. Results show that there is good induction of hypoxia signal across different cell lines and the signal is reversible when the O2 levels go back from 2.5% to 20%.

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



Figures 5A and B: HeLa cells (A) or MCF7 (B) were grown on Nunc™ Sphera 96-well U-bottomed plates for 5 days for formation of spheroids in complete medium. The spheroids were stained with 5 µM Image-iT™ Hypoxia Probe for 3 hr. The images were taken on a Zeiss 710 confocal microscope. **Figure 5C**: HeLa cells were grown on Nanoculture plates (Scivax) for formation of spheroids in complete medium. The spheroids were grown for 6 days and stained with 10 µM Image-iT™ Hypoxia Probe for 1 hr. The images were taken on an EVOS® FLAuto Imaging system.

Conclusion

- Image-it™ Hypoxia Reagent is a novel fluorogenic cell-permeable probe with an excitation and emission spectra of 490 nm and 610 nm respectively (Figure 1).
- Image-it™ Hypoxia Reagent fluoresces at as low as 5% O2 levels and more sensitive when compared to piminidazole adducts that can be detected only at very low O2 levels (Figure 3).
- Image-it™ Hypoxia Reagent is easy to use with a reversible fluorogenic response to O2 levels (Figures 2 and 4).
- Image-it™ Hypoxia Reagent is an ideal tool for detecting hypoxic conditions around tumors, 3D cultures, spheroids (Figure 5),

References

1. Zhang, S., Hosaka, M., Yoshihara, T., Negishi, K., Iida, Y., Tobita, S., & Takeuchi, T. (2010) Phosphorescent light–emitting iridium complexes serve as a hypoxia-sensing probe for tumor imaging in living animals. Cancer Research 70(11), 4490-4498.

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