Application note

MakCell
Compact Automatic Cell Culture Apparatus

Data courtesy of Specially Appointed Associate Prof. Matsui, The University of Osaka.

Publication Date: Sep. 1, 2025

Hypoxic Culture of HeLa Cells Using MakCell

The tabletop automated culture system MakCell is an automated medium exchange device that also supports hypoxic culture. In conventional hypoxic culture, when medium is exchanged manually, cultured cells must be exposed to ambient air even if the incubator itself is under hypoxic conditions. In contrast, MakCell performs medium exchange while maintaining the hypoxic environment, enabling stable cell culture without disturbing the culture conditions (Ref. Application Note "Stability in hypoxic environments of MakCell"). In this study, HeLa cell lines were cultured and analyzed using the hypoxia function of MakCell. Immunofluorescence staining was performed with an antibody against HIF1α, a protein known to be specifically localized in the nucleus under hypoxic conditions. As a result, nuclear-specific localization of HIF1α was observed starting at approximately 1.0 hour of culture.

METHOD

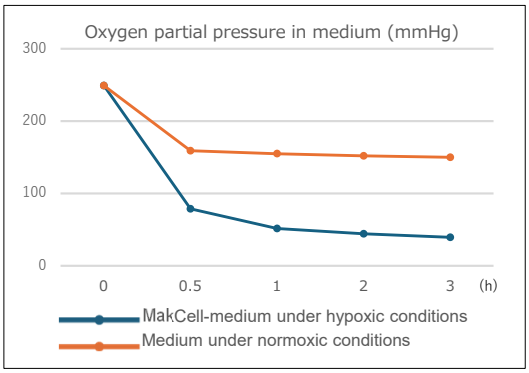
- Cell type: HeLa cell line (seeded at 2 × 10^5 cells/well after maintenance culture in T-75 flasks, followed by trypsinization and seeding into the culture vessels)
- Maintenance medium: DMEM (high glucose, Wako), 10% FBS (Sigma), 1% Anti-Anti (Gibco)
- Culture vessels: 12-well plate (Falcon), φ15 glass (Matsunami)
- Cell counting device: ViCELL MetaFLEX (Beckman Coulter)
- Antibodies: HIF1α antibody (Abcam), fluorescently labeled secondary antibody (ThermoFisher)

Cells were transferred into MakCell set to 1% O_2, then fixed and fluorescently stained at 1, 3, and 6 h.

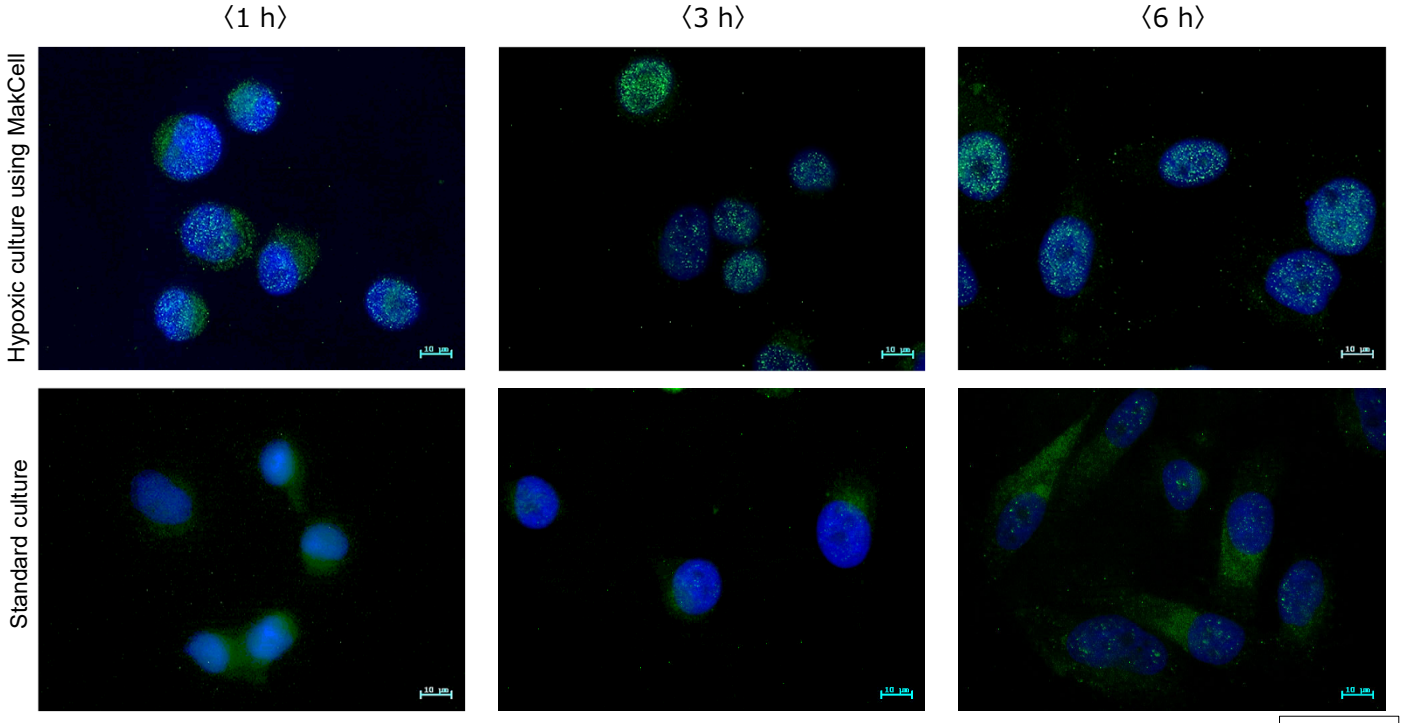
RESULTS

・Measurement of medium inside the incubator conditions:

MakCell culture: CO_2 5%, O_2 1%
Standard culture: CO_2 5%, O_2 19–20%



・**Localization of HIF1α in HeLa cells under each oxygen concentration**

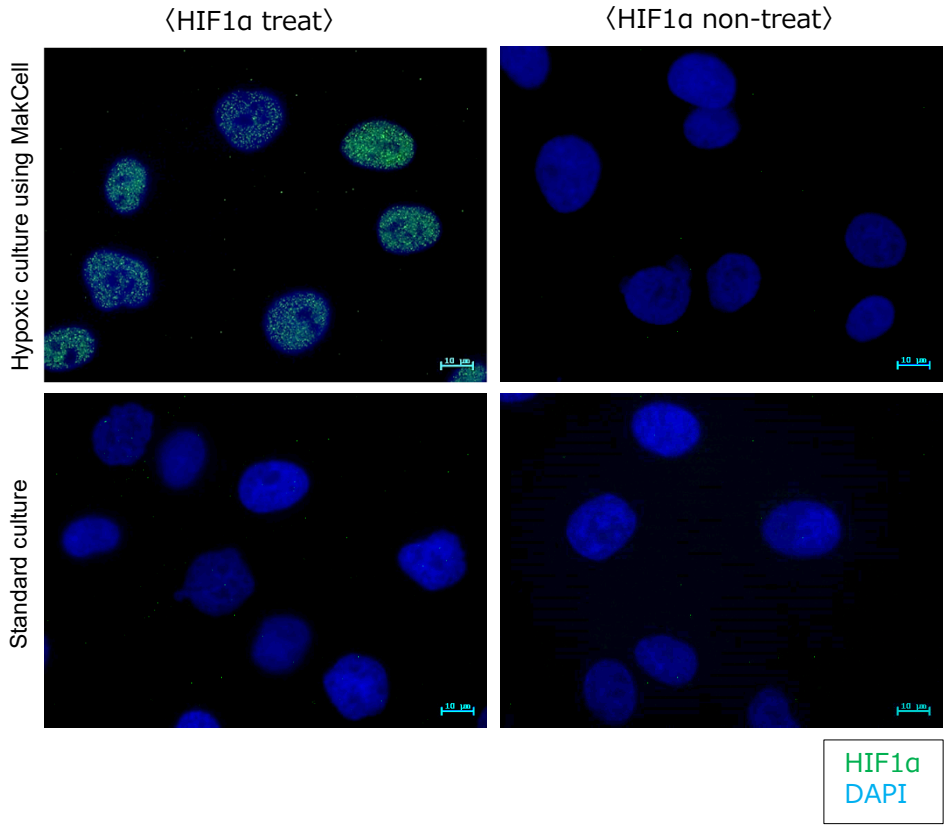


<Reference> Localization of HIF1α in human adipose-derived mesenchymal stromal cells under each oxygen concentration

Conditions:

- MakCell culture: CO_2 5%, O_2 1%
- Standard culture: CO_2 5%, O_2 19–20%

Samples: 3-hour samples under both conditions, using the same culture vessels and equipment as above.



DISCUSSION

- MakCell was set to a hypoxic environment, achieving a hypoxic condition in the medium within just 1 hour and subsequently maintaining a stable state.
- Immunofluorescence staining confirmed that HIF1α translocates to and localizes within the nucleus under a hypoxic condition.
- MakCell demonstrated that it can accommodate experiments measuring the temporal response of HIF1α nuclear localization during transition to a hypoxic environment. This is because, after reaching the set O_2 concentration, fluctuations in O_2 concentration caused by opening and closing the door during storage of the cell culture vessel inside the incubator return to the set value within a relatively short time of 12 minutes (Ref. Application Note "Stability in hypoxic environments of MakCell").
- Long-term culture under hypoxic conditions using MakCell offers the advantage of avoiding O_2 concentration fluctuations and maintaining stability in the cell culture environment, as the automatic medium exchange function eliminates the need to take the culture vessel out from the incubator. We plan to evaluate the usefulness of MakCell for automated medium exchange under hypoxic conditions, including the use of mesenchymal stromal cells, which have been reported to exhibit enhanced proliferation and stem cell marker gene expression under hypoxia.

JTEC CORPORATION
2-5-38 Saito-Yamabuki, Ibaraki, Osaka 567-0086, Japan
Contact
Phone: +81-72-655-2786
Mail: info@j-tec.co.jp