

Stability in hypoxic environments of MakCell

The automated cell culture system, MakCell, can be configured with optional components such as an oxygen gas sensor and a nitrogen gas inlet port. Thereby, this enables automated cell culture under hypoxic conditions. This document presents an evaluation of the stability of the hypoxic environment in MakCell by measuring the dissolved gas partial pressure in the culture medium using a culture medium analyzer and monitoring the internal environment of the system during operation.

ENVIRONMENT

Conventional Incubator: 37°C, CO₂ 5% (Control)

MakCell - Hypoxic ENVIRONMENT: 37°C, CO₂ 5%, O₂ 5%

(Controlled nitrogen gas injection into the chamber at 0.1 MPa)

CONDITIONS

Medium: DMEM (High Glucose, FUJIFILM Wako Pure Chemical), 10% FBS (Sigma),
1% Antibiotic (Gibco)

Equipment: 35 mm dish (BD Falcon)

Medium Volume: 2 mL/dish

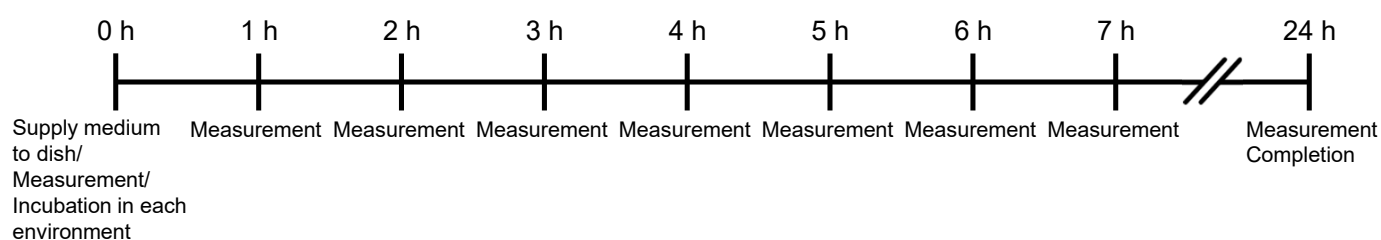
Medium Analysis: Vi-CELL MetaFLEX (Beckman Coulter)

Measured Parameters: pH, pCO₂, pO₂

METHOD

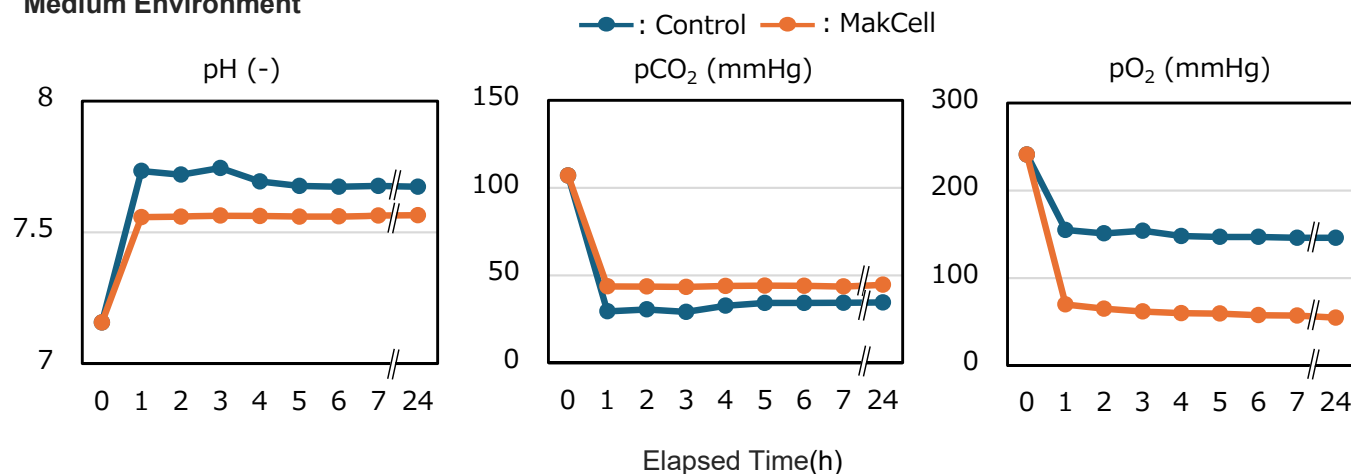
1. Measure the medium components using Vi-CELL MetaFLEX with 2 mL/dish (0 h).
2. Place the dish under each environment and measure the medium components at 1, 2, 3, 4, 5, 6, 7, and 24 h after incubation.
3. Record the time required for the internal environment to reach the set values after opening the door.
4. Operate MakCell and collect data on the internal environment.

Measurement Schedule



RESULTS

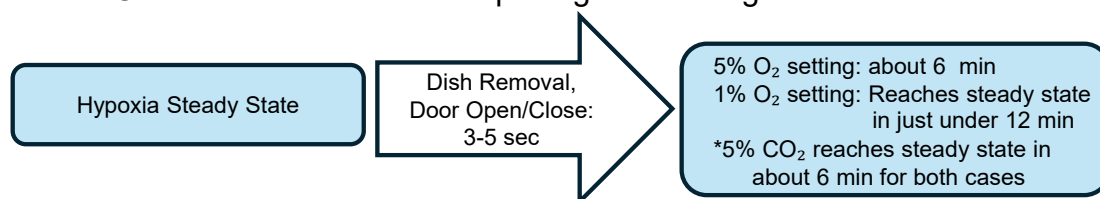
Medium Environment



Oxygen is rapidly released from the medium by nitrogen gas, and the gas pressure stability is high.

RESULTS (continued)

Time for MakCell to stabilize after door opening and closing





➡ Rapid recovery to the set environment

Verification of the stability of the internal gas environment during MakCell operation

【MakCell Setting】

• Temperature 37.0°C • CO₂ 5.0% • O₂ 5.0%

Time	Chamber Temperature (°C)	Chamber O ₂ (%)	Chamber CO ₂ (%)	
9:30:13	37.0	5.0	5.0	
9:35:13	37.0	5.0	5.0	
9:40:13	37.0	5.0	5.0	
9:45:13	37.0	5.0	5.0	
9:50:13	37.0	5.0	5.0	
9:55:13	37.0	5.0	5.0	
9:55:20	MedChange, Exp No:1, Table:1, Pos:7			 Medium change (Set at 9:55) + Nozzle Cleaning
9:57:47	Wash, Exp No:0, Table:1, Pos:1			
10:00:10	37.0	5.0	5.0	
10:00:17	MedChange, Exp No:2, Table:1, Pos:2			 Medium change (Set at 10:00) + Nozzle Cleaning
10:05:00	37.0	5.0	5.0	
10:07:09	Wash, Exp No:0, Table:1, Pos:1			
10:10:08	37.0	5.0	5.0	
10:15:08	37.0	5.0	5.0	
10:20:08	37.0	5.0	5.0	
10:25:08	37.0	5.0	5.0	
10:30:08	37.0	5.0	5.0	

➡ Even during MakCell operation, no fluctuations in chamber temperature or gas concentration were observed.

The chamber environment remained stable before and after operation.

DISCUSSION

MakCell has a compact chamber volume of 140 L, reducing the running cost of hypoxia experiments. Since the culture medium can be exchanged without opening the door, stable long-term hypoxic cultivation is possible.

