CelCradle-500 Technical Report VI

Cultivation of Hi-5 Insect Cells in Ex-CELL 405 Culture Medium

Description

CelCradle-500 provides a powerful tool to achieve high cell density and high productivity of target bioproducts in a cell culture because it has a unique feature of offering high oxygen transfer and low shear stress culture environment. Users can easily collect highly concentrated cells, virus or secreted products from one 500 ml CelCradle-500 bottle. In this study, the application of CelCradle-500 for growth of Hi-5 insect cells in EX-CELL 405 serum-free medium is illustrated. 1.0×10^8 Hi-5 Insect cells were seeded in one CelCradle-500 unit. 53 mg/hr glucose uptake rate can be reached at 5-6 days culture. This technical sheet provides a general protocol for users to start up their culture. However, the optimum condition of each cell culture for each case may require the users to determine.

Material

Device	Cell Line	Medium	Seed
CelCradle-500	Hi-5	EX-CELL 405 (JRH) + 25mM Bis-Tris	1.0 x 10^8 cells/bottle

Protocol

**Please read general instruction manual before starting your culture*

Inoculum Preparation

Prepare one 250ml spinner flask and inoculate 3.0 x 10^5 suspended cells/ml in 120 ml EX-CELL 405 culture media. Culture at 90 rpm, 27°C for 3 days. After cell density reaches above 2.5 x 10^6 cells/ml and viability remain above 95%, it is ready for the preparation of inoculation. Collect 1-1.5x10^8 suspended cells from the spinner flask by centrifugation and collect in one 50ml centrifuge tube with 50ml fresh media.

Preparation before cell seeding

Place CelCradle controller in a 27°C incubator. Setup the inoculation parameters (See below). Warm up EX-CELL 405 medium in 27°C water bath. Take out one CelCradle bottle aseptically and place it in a biosafety cabinet. Open the cap and add 450~470 ml fresh culture medium in the bottle.

Inoculation

Open the cap and dispense 30-50 ml media containing 1-1.5 x 10^8 suspended cells on top of the matrices of CelCradle-500. Bring the bottle and lock up on the CelCradle controller in incubator at 27℃ and start compression immediately. Avoid swirling or shaking the bottle before compression.

Culture

Press “START” button to start the controller. After 2 to 3 hours, reset the parameters for culture condition. Usually, above 90% cells will be immobilized in the matrices within 30 minutes. The inoculation parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
2.0 mm/s	20 sec	2.0 mm/s	0 sec

The culture parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	0 sec	1.5 mm/s	90 sec

The setup parameters are only for reference. It does not necessary to be optimum parameters.

Monitor

Monitor the pH, residual glucose concentration and other metabolites by NOVA Bioprofile 100 analyzer in order to monitor the cell growth. When pH below 6.1 during culture, add Bis-Tris to re-adjust the pH to above 6.5. When glucose concentration below 1.0 g/L, renew the culture medium.

Result

Table 1 shows the time frame to supply Bis-Tris concentrate. Bis-Tris is used for adjust pH value in culture media.

Table 1. Time frame to add buffer concentrates

Culture Time (hours)	pH before adjustment	Add vol. of 1 M Bis-Tris [ml]	pH after adjustment
100.5	6.21	4.8	6.44
117.0	6.15	4.0	6.40

144.0	6.00	10.0	6.37

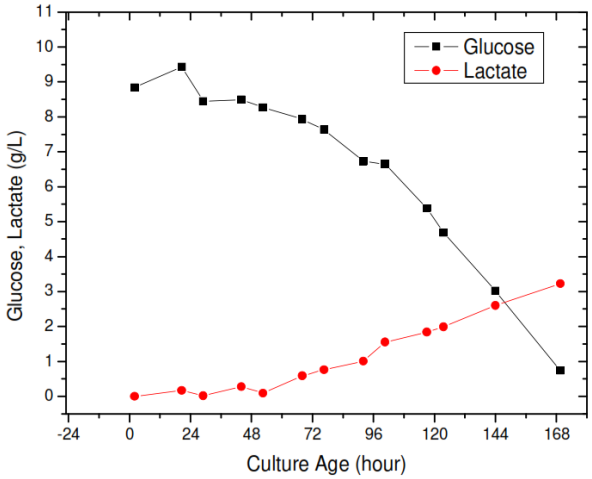

Figure 1: The profile of glucose and lactate concentration change in the culture media.

Glucose was consumed to below 1.0 g after 7 days culture. While lactate concentration continuous to rise to around 3 g/L at the 7th day culture.

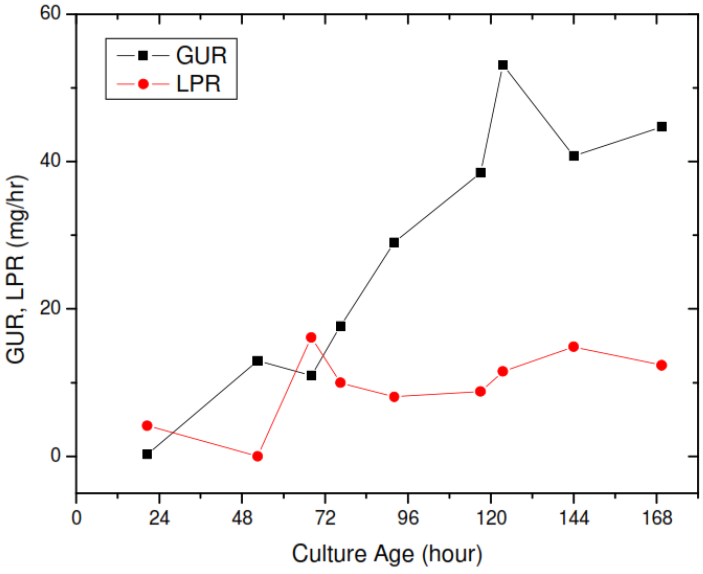

Figure 2: The profile of glucose uptake rate and lactation production rate in mg/hr

Glucose uptake rate reaches the maximum of 53 mg/hr at day 5th. Lactate production rate is keeping at around 10~15 without continuously rising as cell population increased.

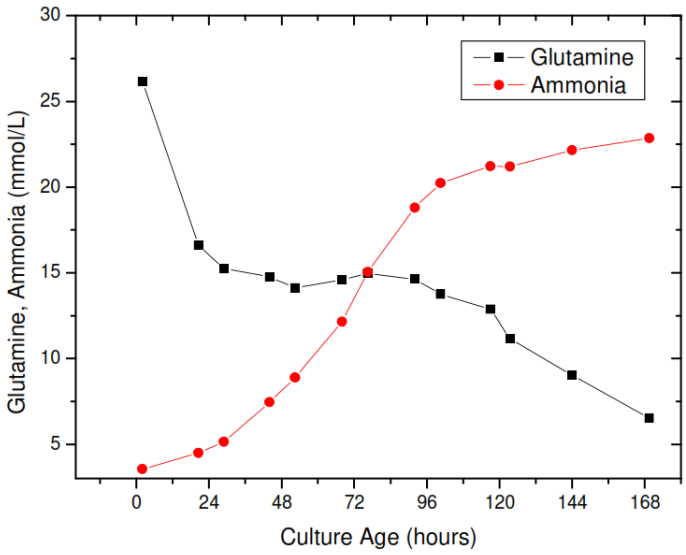

Figure 3: The profile of glutamine and ammonia concentration

The consumption of glutamine was slower down from 24 hours to 96 hours, and then continuously drops. While ammonia accumulation was slow down after reach 20 mmol/L. Generation of ammonia seems not directly come from the consumption of glutamine.

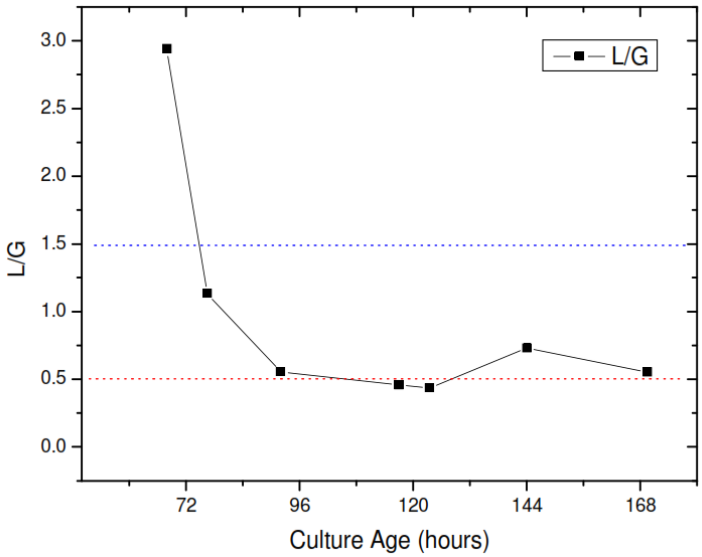

Figure 4: The profile of Lactate generation/Glucose consumption at mol/mol

L/G is around 0.5 mol/mol. This is a good indication of sufficient oxygen supply during culture.

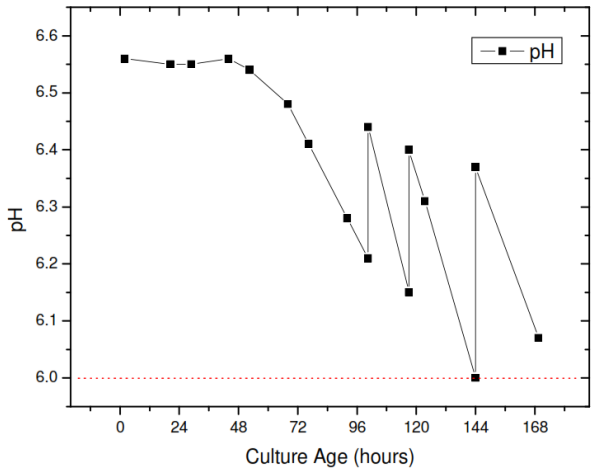

Figure 5: The profile of pH during culture

Three times of Bis-Tris replenishment to control the pH to above 6.0.

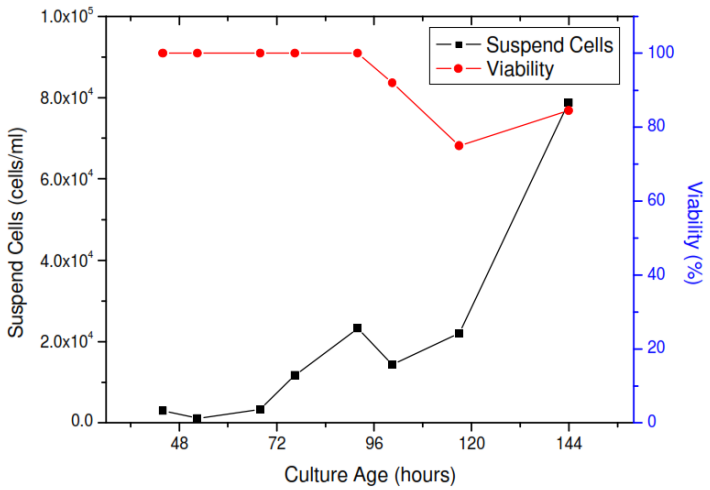

Figure 6: Free suspend cells and viability during culture

Cell viability in supernatant is kept at 100% until day 4 and drop to around 80% at day 5 and day 6. Free suspended cells in culture medium from almost zero, continuously raise to around 8.0 x 10^4 cells/ml. It is still a relatively small amount of cells as compared to the immobilized cells, which is around 2.0 x 10^9 cells at day 6.

The result indicates that CelCradle cell culture system can be applied in Hi-5 insect cell culture for high cell density culture. Total cell population during the culture is around 4×10^9.

Summary

Seed	Inoculum Volume	Medium Volume	Medium
1.0 x 10^8 cells/bottle	50 ml	500 ml	EX-CELL 405
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Max. Cell density or GUR**
169.5 hours	500 ml	0 times	53 mg/hr

Please contact VacciXcell technical support for any questions or comments.

mail@vaccixcell.com

Website: http://vaccixcell.com/