CelCradle-500 Technical Report III

Cultivation of Sf-9 Insect cells in EX-CELL 420 Media

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1 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 Sf-9 insect cells in EX-CELL 420 serum-free medium is illustrated. $2x10^8$ SF-9 Insect cells in one CelCradle-500 unit. 800 mg/day glucose uptake rate can be reached at 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.

2 Material

Device	Cell Line	Medium	Seed
CelCradle-500	Sf-9	EX-CELL 420 (JRH)	2.0 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one 250ml spinner flask and inoculate $3.0x10^5$ suspended cells/ml in 120ml EX-CELL 420 culture media. Culture at 90 rpm, 28°C for 4 days. After cell density reaches above $1.5x10^6$ cells/ml and viability remain above 95%, it is ready for the preparation of inoculation. Collect $2.0x10^8$ suspended cells from the spinner flask by centrifugation and collect in one 50ml centrifuge tube with 50ml fresh media.

3.2 Preparation before cell seeding

Place CelCradle controller in a 28℃ incubator. Set up the inoculation parameters (See below). Warm up EX-CELL 420 medium in 28℃ waterbath. 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.

3.3 Inoculation

Open the cap and dispense 30-50 ml media containing $2.0x10^8$ suspended cells that has been prepared previously on top of the matrices of CelCradle-500. Bring the bottle and lock up on the CelCradle controller in incubator at 28℃ and start compression immediately. Avoid swirling or shaking the bottle before compression.

3.4 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
2.0 mm/s	10 sec	2.0 mm/s	1 min 30 sec

Monitor the pH, residual glucose concentration and other metabolic in order to predict the time for virus infection or the termination of culture.

When pH below 6.0 during culture, add Bis-Tris to re-adjust the pH to above 6.5. There is no need to replenish media at this application.

4 Result

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

Table 1. Time frame to add concentrates

Culture Time (Hours)	pH	1 M Bis-Tris (ml)	1.67 M Glucose concentrate (ml)
69.5	6.08	1.0	
102.5	6.31	1.5	
127.5	6.09	2.0	
145.6	6.26	4.5	
167.5	6.24		4.0
190.5	6.02	3.0	

Figure 1 shows the glucose and lactate concentration change in the culture media.

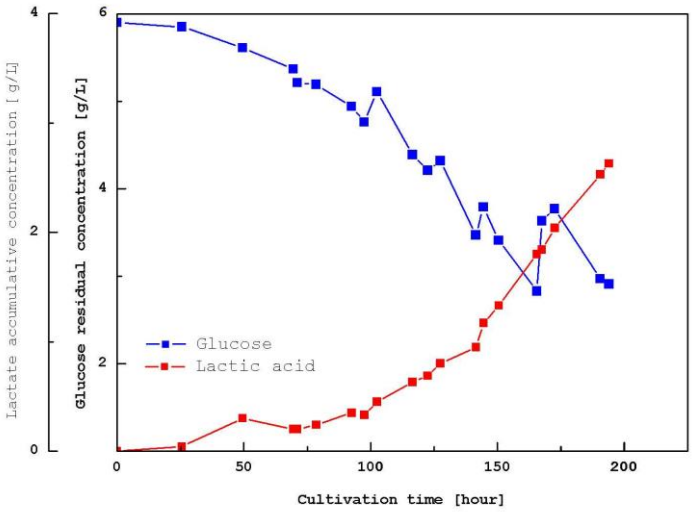

Figure 1: The profile of glucose and lactate concentration

Figure 2 shows the profile of glucose uptake rate(GUR) during culture.

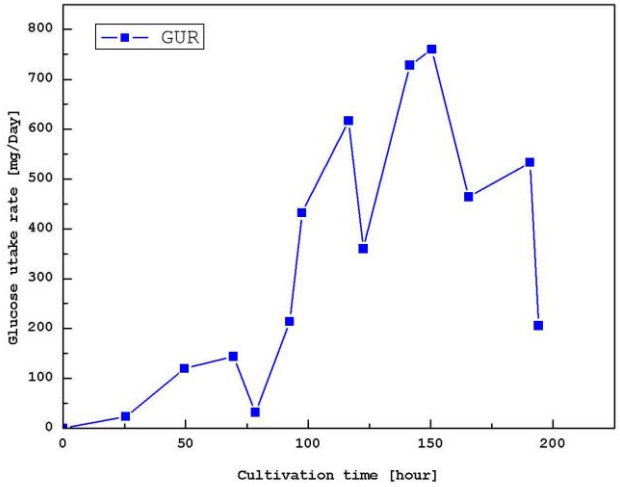

Figure 2: The profile of glucose uptake rate in mg/day

The result indicates that CelCradle cell culture system can be applied in Sf-9 insect cell culture for high cell density culture without the requirement to replenish media.

5 Summary

Seed	Inoculum Volume	Medium Volume	Medium

2.0 x 10^8 cells/bottle	50 ml	500 ml	EX-CELL 420
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Max. Cell density or GUR**
190.5 hours	500 ml	0 times	800 mg/day

6 VacciXcell Technical Support

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