CelCradle-500 Technical Report II

Cultivation of Sf-9 Insect cells in SF 900 II 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 is illustrated. 1.5×10^8 SF-9 Insect cells were seeded in one CelCradle-500 unit. A final 6.3×10^9 cell population in one CelCradle-500 unit was achieved. A total 48.7 folds of cell expansion was achieved within 8 days culture period. 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 Materials

Device	Cell Line	Medium	Seed
CelCradle-500	Sf-9	SF900 II (Gibco, Invitrogen)	$1.5x10^8$ cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one 250 ml spinner flask and inoculate 2.5×10^5 suspended cells/ml in 130 ml SF 900 II culture media. Culture at 90 rpm, 28℃ for 3 days. After cell density reaches above 1.5×10^6 cells/ml and viability remain above 95%, it is ready for the preparation of inoculation. Collect 1.5×10^8 to 2.0×10^8 suspended cells from the spinner flask by centrifugation and collect in one 50 ml centrifuge tube with 50 ml fresh media.

3.2 Preparation before cell seeding

Place CelCradle controller in a 28℃ incubator. Set up the inoculation parameters (See below). Warm up SF900 II medium in 28℃ water bath. Take out one CelCradle bottle aseptically and place it in a biosafety cabinet. Open the cap and add 450 ml fresh culture medium in the bottle.

3.3 Inoculation

Open the cap and dispense 50 ml media containing $1.5x10^8$ to $2.0x10^8$ suspended cells on top of the matrices of CelCradle-500. Bring the bottle and lock up on the CelCradle console immediately in incubator at 28℃ and start the run 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.

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

4 Result

Table 1 shows the monitored glucose concentration and calculated GUR, where GUR is calculated by:

$$GUR\ (\frac{mg}{day}) = \frac{\left(glucose\ at\ time\ 2\left(\frac{mg}{l}\right) - glucose\ at\ time\ 1(\frac{mg}{l})\right)}{(time\ 2(hr) - time\ 1(hr))} x\ Medium\ Volume\ (L)\ x\ 24\ (hrs)$$

One can also use mg/hr for the unit of GUR without timing 24.

Table 1. Time frame to add concentrates.

Culture Time		Glucose	GUR
hour	day	g/L	Mg/day
0.0	0.00	9.800	-
72.0	3.00	9.430	61.667
97.3	4.05	8.939	233.810
120.0	5.00	8.701	125.263
168.0	7.00	6.250	811.500

192.0	8.00	4.516	867.000

Figure 1 shows the glucose concentration change in the culture media and the glucose uptake rate, which is an indication of cell population.

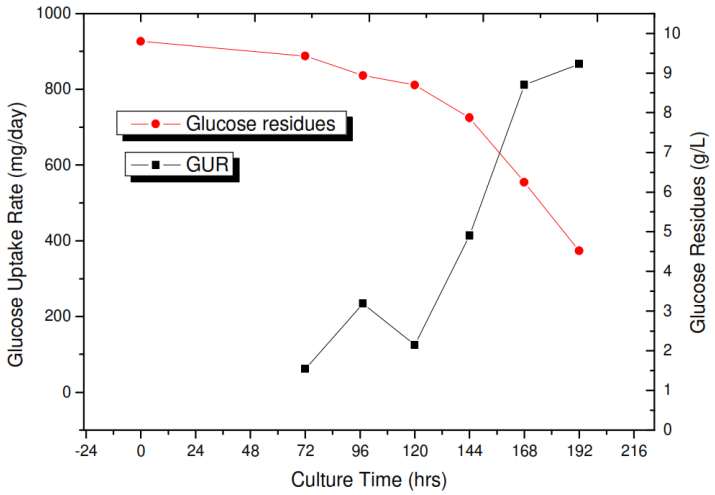

Figure 1: The profile of glucose concentration and glucose uptake rate in CelCradle-500 unit. Total cells counted by crystal violet nucleus dye is 6.3 x 10^9 cells/bottle.

After cells grow and occupy all the space in the matrices, free suspend cells will start to increase in the culture media. Below is the suspend cell density curve during culture period. It indicates that cells can be immobilized in matrices with high efficiency until the end of culture.

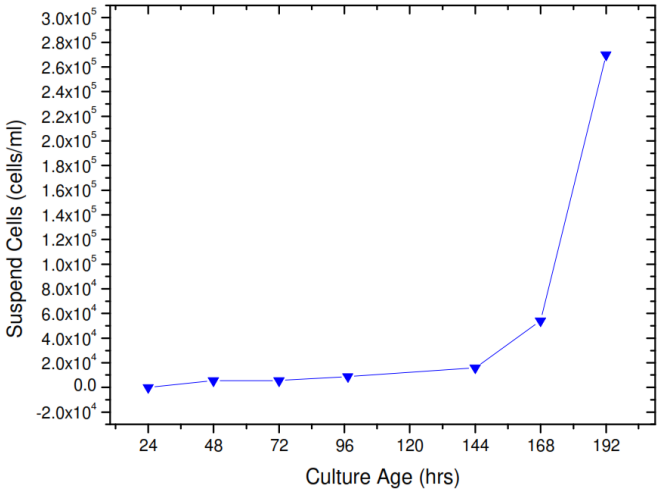

Figure 2: The profile of suspend cell concentration in CelCradle-500 unit. Before 7 days culture, free suspended cell density is below 6 x 10^4, which is about 0.4% of the total cell population.

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
1.5 x 10^8 cells/bottle	50 ml	500 ml	SF900 II
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Final Cell Density**
192 hours	500 ml	0 times	6.3 x 10^9 cells/bottle

6 VacciXcell Technical Support

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