CelCradle-500 Technical Report XVII

Cultivation of Sf-21 Insect cells in TNM-HF/5%FBS 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 culture medium is illustrated. 2.5×10^8 for growth of Sf-21 insect cells in TNM-FH/5%FBS SF-21 Insect cells were seeded in one CelCradle-500 unit. A total of 16.5 folds increase and 4.3×10^9 cells can be reached at 9 days culture by consuming 1.5 L culture medium. 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-21	TNM-FH/5%FBS	2.5×10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one 250 ml spinner flask and inoculate 3.0×10^5 suspend cells/ml in 120 ml TNM-HF/10%FBS culture media. Culture at 75 rpm, 28℃ for 4 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 2.5×10^8 suspend cells from the spinner flask by centrifugation and collect in one 50 ml centrifuge tube with 30 ml fresh media.

3.2 Preparation before cell seeding

Place CelCradle Stage controller in a 28℃ incubator. Set up the inoculation parameters (See below). Warm up TNM-HF/5%FBS medium in 28℃ water bath. Take out one CelCradle bottle aseptically and place it in a biosafety cabinet. Open the cap and add 470 ml fresh culture medium in the bottle.

3.3 Inoculation

Open the cap and distribute 30 ml media containing 2.5×10^8 suspended cells that has been prepared previously on top of the matrix of CelCradle-500. Bring the bottle and lock up on the CelCradle Stage controller 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 3 hours, reset the parameters for culture condition. Usually, above 90% cells will be immobilized in the matrices within 30minutes. 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

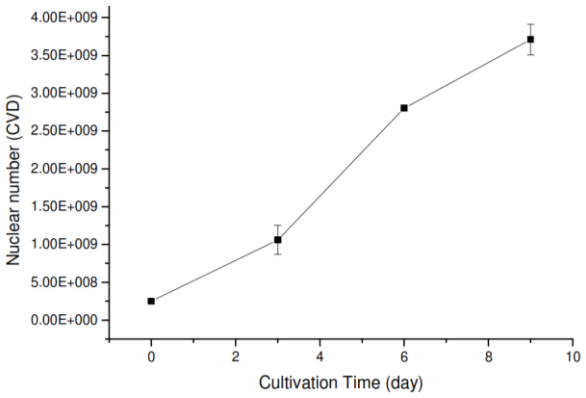
The culture parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
2.0 mm/s	0 sec	2.0 mm/s	1 min 30 sec

Due to there are only 0.7 g/L glucose in the culture medium, the pH is very stable during whole culture period. Culture medium were replenished every 3 days and carrier samples were took to count and observe the cell growth.

4 Result

Cell Growth Profile



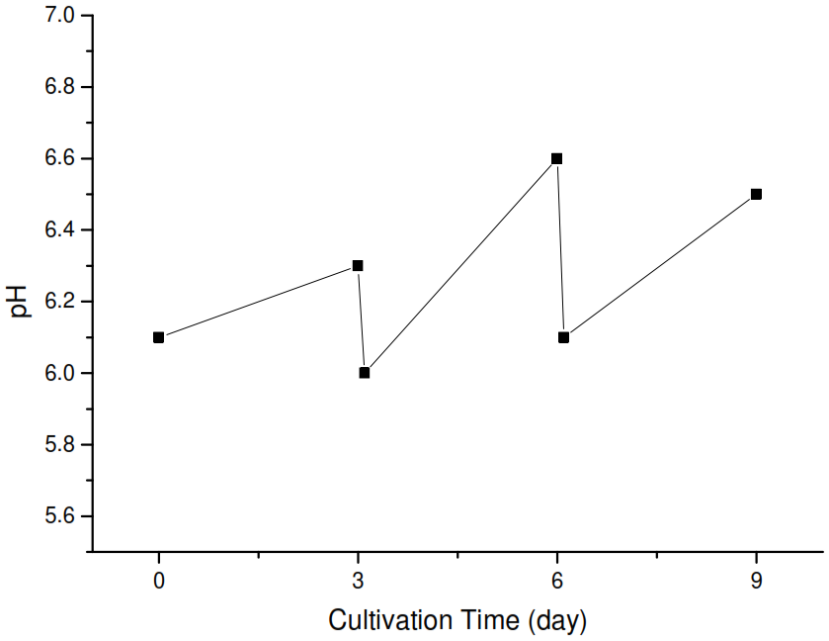
The cell density started from 2.5 x 108 and reached 4.3 x 109 with 9 days culture. The growth rate start to slow down after the 6th day, which means the medium exchange frequency should be increased from every 3 days to every 2 days.

Cell Count on carriers

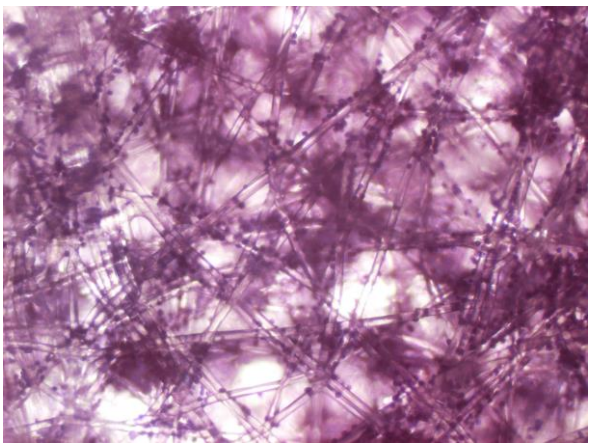
Table 1. The cell number on carriers (CVD method)

Cultivation Time		CVD Nuclear Count		
Days	Hours	2-3 samples raw data	Average	SE
0	0	*	2.5×10^8	0
3	76.5	8.65×10^8	1.06×10^9	1.93×10^8
		1.25×10^9		
6	148	2.80×10^9	2.80×10^9	0
		2.80×10^9		
8	217	4.08×10^9	3.71×10^9	2.03×10^8
		3.38×10^9		
		3.68×10^9		

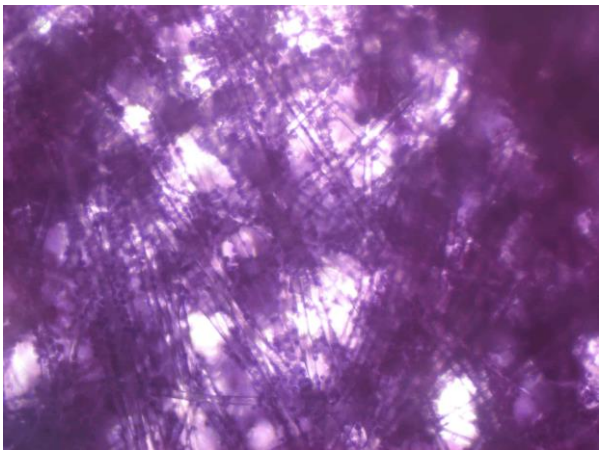
pH



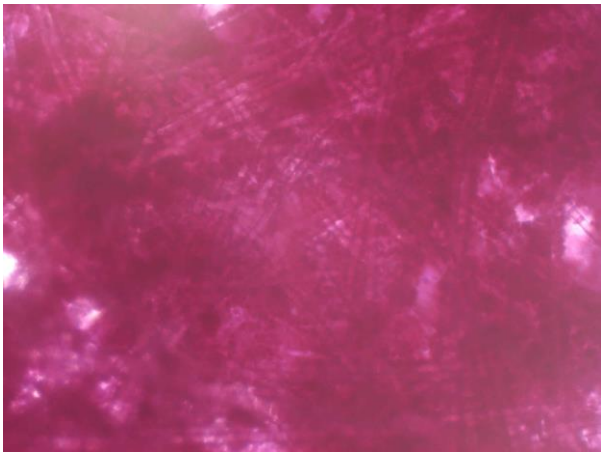
pH is stable and even raise during culture. Maintenance of culture is straightforward.



Cells at Day 3



Cells at Day 6



Cells at Day 9

The result indicates that CelCradle cell culture system can be applied in Sf-21 insect cell culture for high cell density culture with very little care on pH control and nutrient supply.

5 Summary

Seed	Inoculum Volume	Medium Volume	Medium
2.5 x 10^8 cells/bottle	30 ml	500 ml	TNF-FH/5%FBS
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Max. Cell Density**
217 hours	1.5L	2 times	4.33 x 10^9 cells/bottle

6 VacciXcell Technical Support

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