• BelloCell-500 Technical Report II •

Cultivation of Sf-9 Insect cells in SF 900 II media

Description BelloCell-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 BelloCell-500 bottle. In this study, the application of BelloCell-500 for growth of Sf-9 insect cells is illustrated. 1.5×10^8 SF-9 Insect cells were seeded in one BelloCell-500 unit. A final 6.3×10^9 cell population in one BelloCell-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.

Material

Device	Cell Line	Medium	Seed
BelloCell-500	SF-9	SF900 II (Gibco, Invitrogen)	1.5×10^8 cells/bottle

Protocol **Please read General Instruction Manual before start your culture*

Inoculum preparation Prepare one 250 ml spinner flasks and inoculate 2.5 $\times10^5$ suspend 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 suspend cells from the spinner flask by centrifugation and collect in one 50 ml centrifuge tube with 50 ml fresh media.

Preparation before cell seeding Place BelloStage-3000 controller in a 28℃ incubator. Set up the inoculation parameters (See below). Warm up SF900 II medium in 28℃ water bath. Take out one BelloCell bottle aseptically and place it in a biosafety cabinet. Open the cap and add 450 ml fresh culture medium in the bottle.

Inoculation Open the cap and dispense 50 ml media containing 1.5×10^8 to 2.0×10^8 suspend cells that has been prepared previously on top of the matrix of BelloCell-500. Bring the bottle and lock up on the BelloStage console immediately in incubator at 28℃, 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
2.0 mm/s	10 sec	2.0 mm/s	1min 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 during culture stage. *The setup parameters are only for reference. It does not necessary to be optimum parameters. Cesco will update recent data on the website, please check the website for updated information.*

Result

Table 1 shows the monitored glucose concentration and calculated GUR, where GUR is calculated by (glucose at time 2(mg/L) - glucose at time 1)/(time2(hr)-time1)×media volume(L) ×24 (hrs) with unit: mg/day. One can also use mg/hr for the unit of GUR without timing 24.

Table 1. Culture metabolites and glucose uptake rate (GUR)

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
144.0	6.00	7.873	414.000
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.

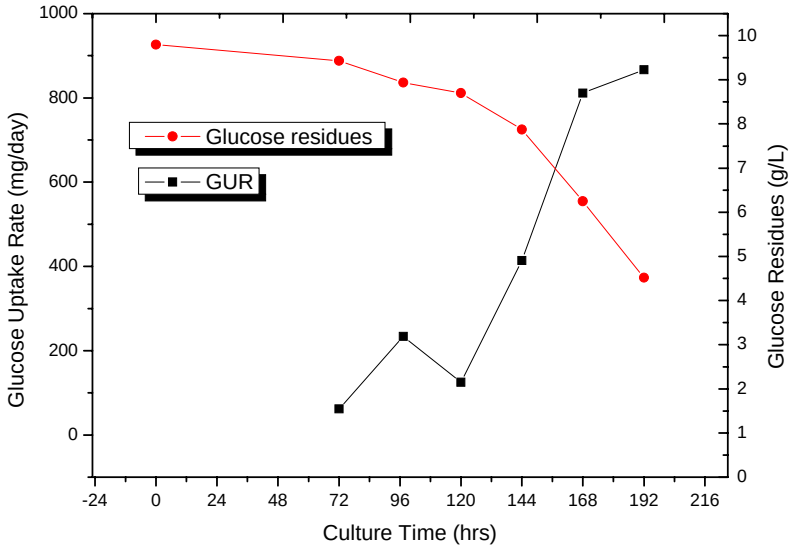

Figure 1: The profile of glucose concentration and glucose uptake rate in BelloCell-500 unit. Total cells counted by crystal violet nucleus dye is 6.3× 10^9 cells/bottle.

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.

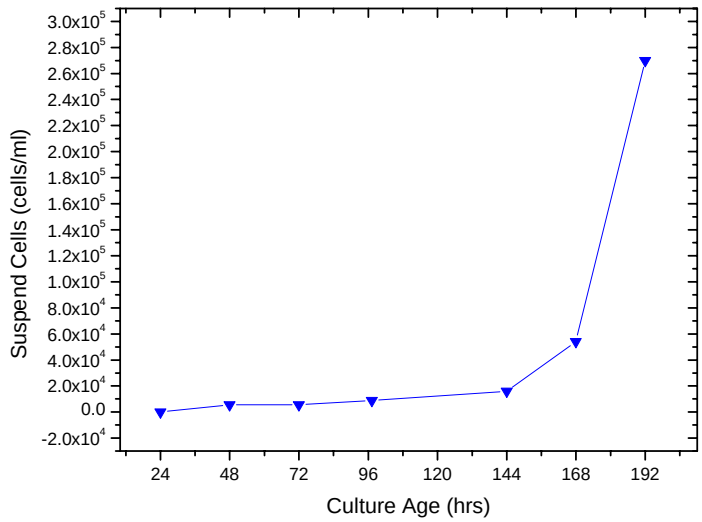

Figure 2: The profile of suspend cell concentration in BelloCell-500 unit. Before 7 days culture, free suspend cell density is below 6×10^4 , which is about 0.4% of the total cell population.

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

Summary

Seed	**Inoculum volume**	**Medium volume**	**Medium**
1.5×10^8 cells/bottle	50 ml	500 ml	SF900 II
Total culture age	**Total medium consumed**	**Total medium replenish frequency**	**Final cell density**
192 hrs	500 ml	0 times	6.3×10^9 cells/bottle

Please contact Cesco Bioengineering Technical support for any questions or comments.

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