• BelloCell-500 Technical Report IV •

Cultivation of Sf-9 Insect cells and baculovirus expression system in SF 900 II media *

* This experiment has been published in Genetic Engineering News Issue Sep. 01, 2003

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 and production of baculovirus and enhanced GFP protein is illustrated. Three culture systems, i.e. spinner flask, spinner basket, and BelloCell, were compared simultaneously. 1.5×10^{8} SF-9 Insect cells were seeded in one BelloCell-500 unit. The cells were infected by BAE baculovirus with a MOI of 10. A total of 3.45×10^{12} baculovirus and 14,355 unit of e-GFP protein was produced in one BelloCell unit. e-GFP protein production in BelloCell system is 1.8 folds higher than a spinner basket and 7.9 folds higher than a 250 ml spinner flask. 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, Spinner flask (Corning), Spinner basket (NBS)	SF-9	SF900 II (Gibco)	3×10^{5} cells/ml
Virus	**MOI**		
BacCE baculovirus*	10		

* BacCE baculoviruses are kindly provided by Dr. Hu in Tsing-Hua University, Taiwan

Protocol for BelloCell system

Inoculum preparation Prepare one 250 ml spinner flasks and inoculate 2.5×10^{5} suspend cells/ml in 120 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} 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 control 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 well distribute 50 ml media containing 1.5×10^8 suspend cells on top of the matrixes of BelloCell-500. Bring the bottle and lock up on the BelloStage controller in incubator at 28℃.

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 during culture stage. *The setup parameters are only for reference. It does not necessary to be optimum parameters.*

Infection When the growth reach mid-log phase, where GUR is 25 mg/hr, prepare for virus infection. Replace the culture medium and Infect cells with MOI=10. Control the pH by adding Bis-Tris to above 6.5.

Result

Below shows the result of cell growth, cell density, virus production, and E-GFP protein production in the BelloCell-500 system. Figure 1 shows the profile of glucose uptake rate in three different culture systems. BelloCell-500 provides optimum environment for cells to grow up to 1.4×10^7 cells/ml, while other two systems have relative lower cell density.

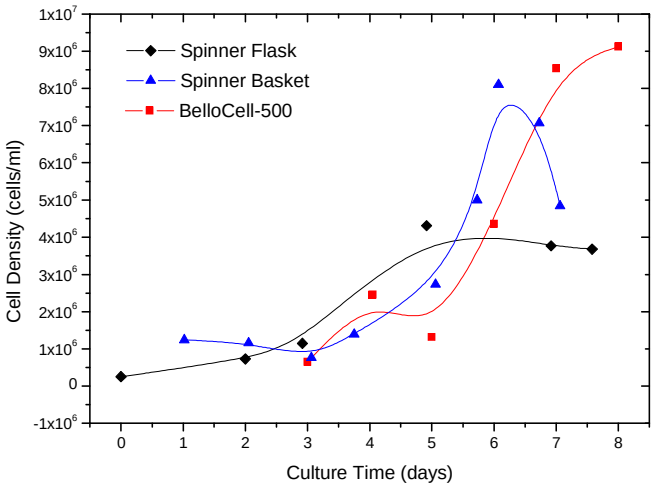

Figure 1. profile of cell density in three different culture systems

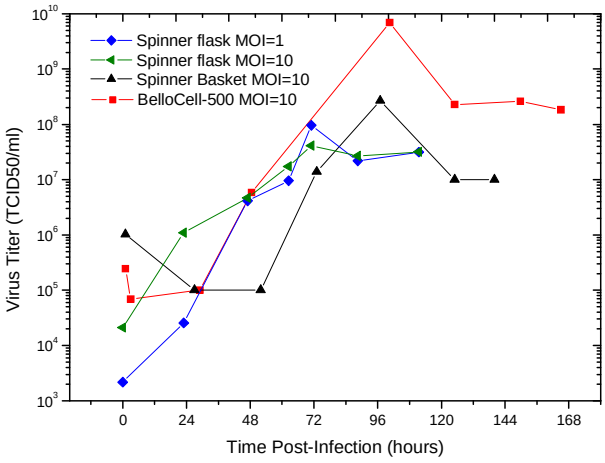

Figure 2. The baculovirus production in three different culture system. Virus productivity in BelloCell-500 is 30 folds higher than spinner basket system and 240 folds higher than spinner flask system.

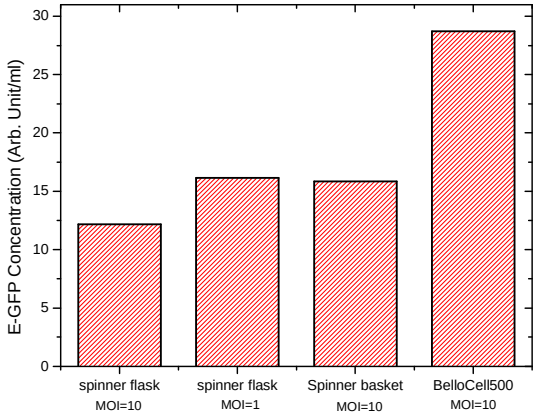

Figure 3: The E-GFP productivity in three different system. Protein concentration in BelloCell-500 is about 2 folds higher compared with other systems.

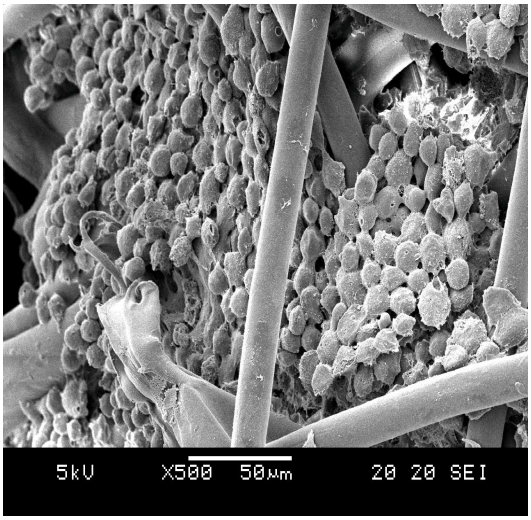

Figure 4: The SEM photo of S-9 cells cultivated in BioNOC II matrix in BelloCell-500 system

The result indicates that BelloCell cell culture system can be applied in Sf-9 insect cell culture for high cell density culture and high viruses and protein production.

Summary

Below is the summary of the comparison of sf-9 cell growth and baculovirus/protein production in various lab culture systems.

	Spinner flask	Spinner Basket/FibraCel disk	BelloCell/BioNOC matrices
Description	250 ml flask/150 ml medium	1000 cm^3 bottle /450 ml medium/100 cm^3 (10 g) carriers	1000 ml bottle/500 ml medium/100 cm^3 (6.5 g) carriers
Total surface area (cm^2)	None	12,000	13,000
Mode of operation	Batch	Batch	Batch
Medium for growth/infection	SF 900 II	SF 900 II	SF 900 II
Max. cell density (cells/ml of medium)	4.3×10^6	8.1×10^6	1.4×10^7
Total cell number	6.5×10^8	3.6×10^9	7.2×10^9
Multiplicity of infection (MOI)	1 and 10	10	10
Virus productivity ($TCID_{50}$/ml)	9.6×10^7	2.6×10^8	6.9×10^9
Total virus production ($TCID_{50}$)	1.44×10^{10}	1.17×10^{11}	3.45×10^{12}
E-GFP (arb. U/ml)	16.13 (MOI=1) and 12.16 (MOI=10)	17.62	28.71
Total E-GFP (arb. U)	2419.6 (MOI=1) and 1824.03 (MOI=10)	7927	14355

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

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