CelCradle-500 Technical Report VI

Cultivation of CHO Cells for Protein Secretion

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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 applications of CelCradle-500 for growth of suspend CHO cells and production of human IgG is illustrated. 7.9 $\times 10^7$ CHO cells were seeded in one CelCradle-500 unit. A final 4.5 x 10^9 cell population in one CelCradle -500 unit was obtained. For the protein production, a total of 157.5 mg IgG protein was harvested in 5 L conditioned culture media. In comparison with 1.1 mg IgG protein in a 200 ml spinner flask. It is 5.7 folds productivity increase on an equal volume basis. 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/Product	Medium	Seed
CelCradle-500	CHO/IgG	EX-CELL 301 (JRH)	7.9 x 10^7 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one 250ml spinner flask and inoculate 1.8 x 10^5 suspended cells/ml in 100ml EX-CELL 301 culture media. Culture at 50 rpm, 37°C for 3 days. After cell density reaches above 8.0x10^5 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 37℃ incubator. Set up the inoculation parameters (See below). Warm up EX-CELL 301 medium in 37℃ waterbath. 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 5 x 10^7 to 1.0 x 10^8 suspended cells on top of the matrices of CelCradle-500. Bring the bottle and lock up on the CelCradle controller in incubator at 37℃ and start compression immediately. Press “START” button to start the controller. Avoid swirling or shaking the bottle before compression.

3.4 Culture

The inoculation parameters were 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

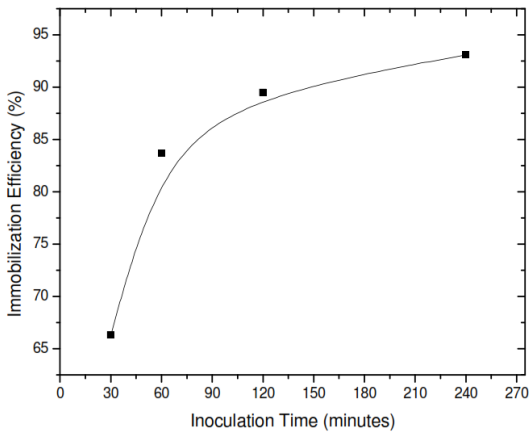
After 2 to 3 hours, reset the parameters for culture condition. Usually, above 90% cells will be immobilized in the matrices within 2 hours. The culture parameters were set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	0 sec	1.5 mm/s	1 min

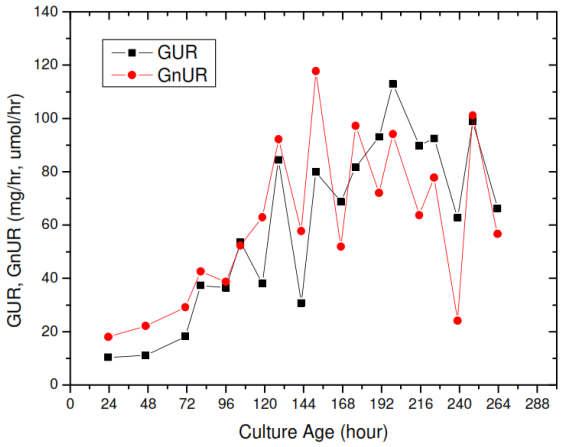
For optimal culture, monitor the pH, residual glucose concentration and other metabolic in order to predict the time for medium replenishment. When pH drop below 7.0 during culture, add 5 ml 1M HEPES and/or 10 ml 7.5% $NaHCO_3$ re-adjust the pH to above 7.5. When glucose concentration below 1.5 g/L, add 5 ml 18 w/w% glucose and 125 mM glutamine concentrate. Replenish culture medium once a day from the day 3 of culture. Add glucose, glutamine, HEPES and $NaHCO_3$ supplements once a day from the day 5 of culture. We suggest that users can replenish culture medium in the afternoon, and add supplement the next day in the morning. For simpler operation, user can replenish medium twice a day in the morning and in the afternoon.

4 Result

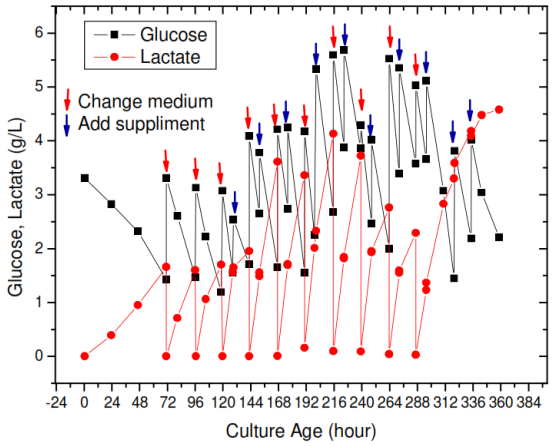
Immobilization Efficiency



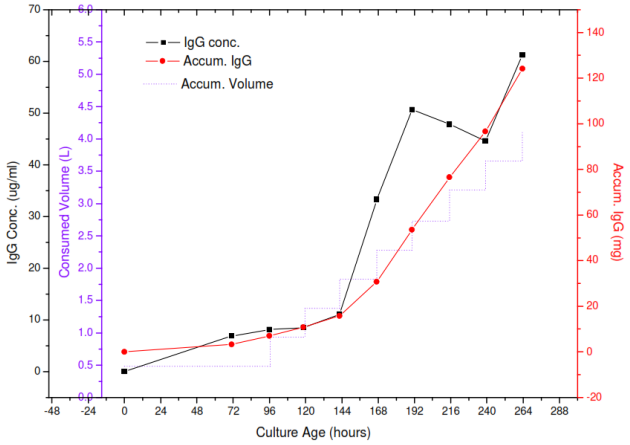
Glucose uptake rate (mg/hr) and glutamine uptake rate (umol/hr)



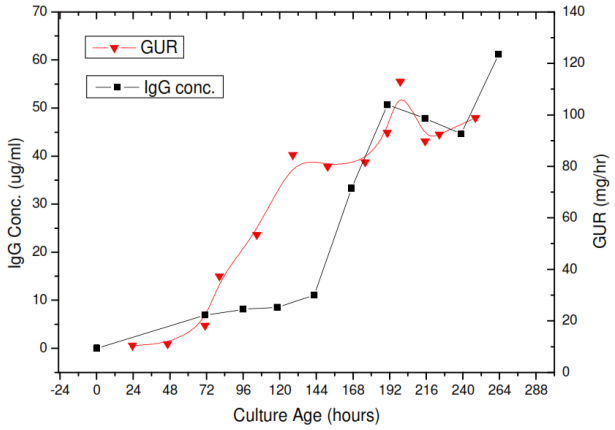
Glucose and Lactate concentration



IgG Production

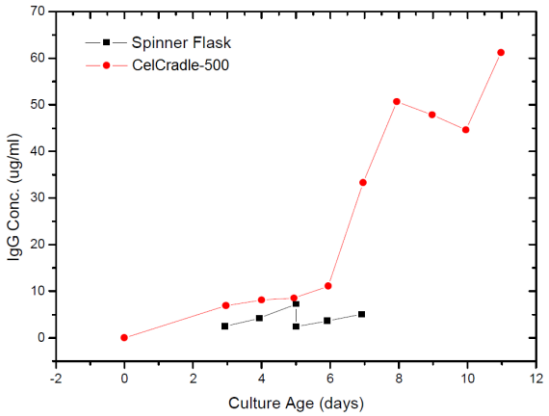


IgG and GUR



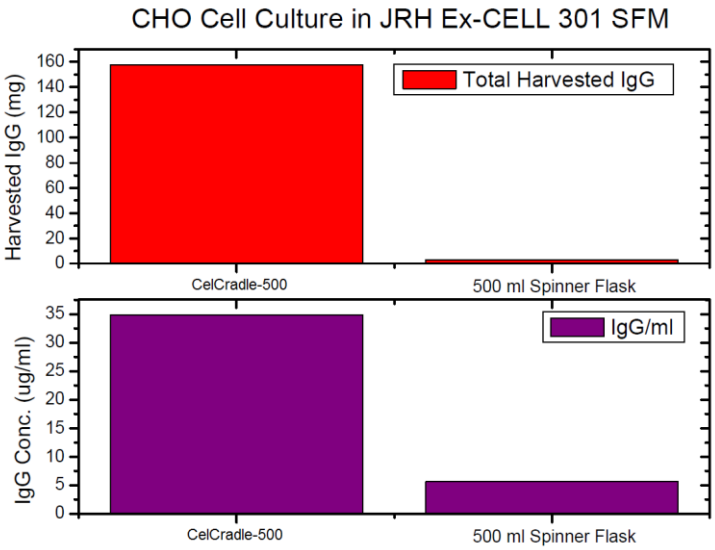
GUR shows a good correlation with IgG production

IgG concentration between CelCradle-500 and Spinner flask



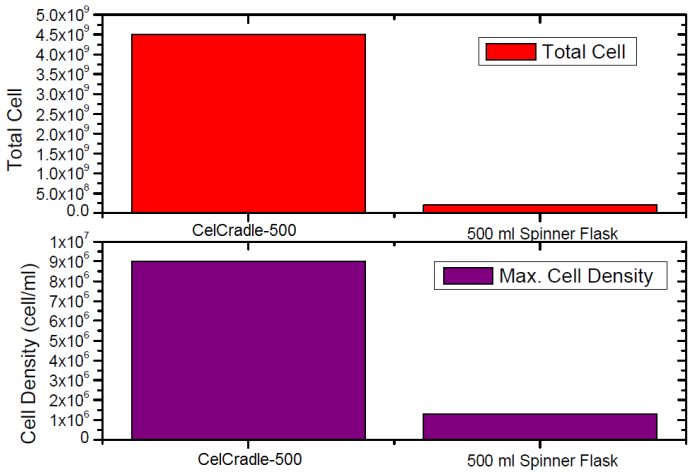
Max. IgG concentration in CelCradle-500 is about 6 folds compared with spinner flask

IgG production comparison with spinner flask



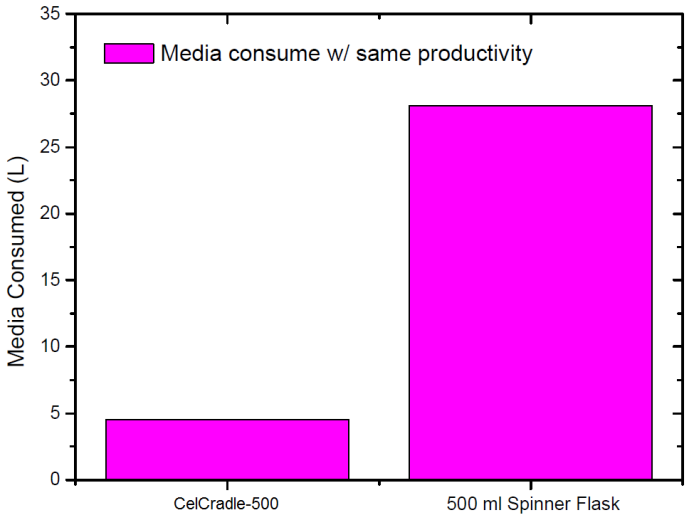
Total harvest IgG in CelCradle-500 is 71 folds compared with a 500 ml spinner flask. Average IgG concentration in CelCradle-500 is around 7 folds compared with a 500 ml spinner flask.

Total cell population and cell density based on same volume



Total cell population in CelCradle-500 is 7.5 folds compared with a 500 ml spinner flask; cell density in CelCradle-500 is 8.2 folds compared with a 500 ml spinner flask.

Medium consumption based on same productivity



For the same production, it will consume 28 L culture medium in a 500 ml spinner to get the same productivity as one CelCradle-500 bottle.

5 Summary

Seed	Inoculum Volume	Medium Volume	Medium
7.9 x 10^7 cells/bottle	50 ml	500 ml	EX-CELL 301
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Total Cell Counted**
15 days	5000 ml	9 times	4.5 x 10^9 cells
Max. GUR	**Total Protein Produced**	**Max. Protein Concentration**	**Multiplication of cells**
121.71 mg/hr	157.5 mg	61.2µg/ml	57 fold

6 VacciXcell Technical Support

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