CelCradle-500 Technical Report XIV

Long-Term Culture of CHO cells for protein secretion in HyQ-PF-CHO medium

Description

CelCradle-500 provides a powerful cell culture tool to achieve high cell density and high productivity of target bioproducts because of its 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-adapted CHO cells and production of human IgG with different medium conditions were illustrated. 2 × 10^8 CHO cells were seeded in each CelCradle-500 unit. A final cell population from 3.5 – 6 × 10^9 in one CelCradle -500 unit was obtained during 35 days culture. For the protein production, a total of 800 mg to 1197 mg IgG protein in different setting was harvested within 35 days culture by consuming 8.0-15.0 L culture medium. At the later stage of culture, approximately 200 mg IgG could be produced in 500 ml culture medium every two days. Comparing with other culture systems, it may require sophisticate control or large volume in order to achieve the goal. This is a standard example for non-growth associated production in many cases such as CHO and hybridoma. This technical sheet provides a general protocol for users to start up their culture. However, the optimum condition of different cell culture may require users to determine.

Material

Device	Cell Line/Product	Medium	Seed
CelCradle-500	CHO/IgG (has been adapted for suspension culture in spinner flask)	HyQ-PF-CHO (Hyclone)	2 x 10^8 cells/bottle

Protocol

**Please read general instruction manual before starting your culture*

Inoculum Preparation

Prepare 2 1000ml spinner flasks. Seed with 2 x 10^5 suspended cells/ml in 2 x 500ml HyQ-PH-CHO culture medium. Culture at 50 rpm, 37℃ for 3 days. After cell density reaches above 1×10^6 cells/ml and viability remain above 95%, it is ready for the preparation of inoculation. Collect 8.0 × 10^8 suspended cells from the spinner flask by centrifugation and separate into four 50 ml centrifuge tubes with each 50 ml fresh media.

Preparation before cell seeding

Place 3 CelCradle Stage controllers in 37℃ incubators. Set up the inoculation parameters (See below). Warm up HyQ-PF-CHO medium in 37℃ water bath. Take out three CelCradle-500 bottles aseptically and place it in a biosafety cabinet. Open the cap and add each bottle with 450 ml fresh culture medium in the bottle.

Inoculation

Open the cap and distribute 50 ml media containing 2.0 x 10^8 suspend 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 37℃. Press "START" button to start the controller. At the same time seed the same cell density in a 1000 ml spinner flask containing 500 ml culture medium for comparison study.

Culture

After 4 hours, reset the parameters for culture condition. Usually, above 90% cells will be immobilized in the matrices within 4 hours. 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 for each bottle are set the same as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.0 mm/s	0 sec	1.0 mm/s	30 mins

During the culture, pH, residual glucose and glutamine concentration and other metabolites were monitored each day, but no intent of control of the glucose and glutamine except pH. pH was controlled by adjusting CO2 concentration in CO_2 incubator and/or increasing $NaHCO_3$ concentration in the fresh medium. For convenience of operation in this study, the cultures were replenished with fresh medium once a day or once per two days from the 3rd day until the end of run. $NaHCO_3$ concentration had been increased from 2.2 g/L up to 3.7 g/L or 4.4 g/L during the run. All culture were started to exchange culture medium by day 3. CO_2 concentration was adjusted from 5% to zero directly by day 3. Replenish culture medium once a day or twice a day from the day 5 of culture.

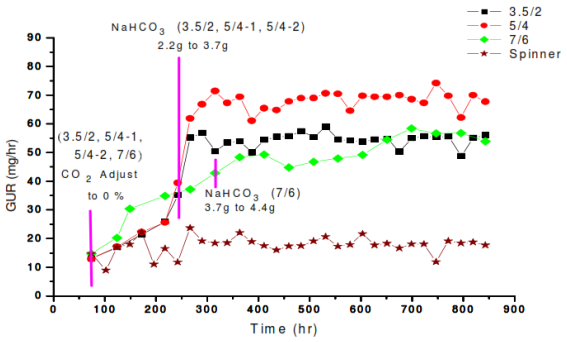
The culture conditions for each bottle are as below:

No.	Glucose Conc.	Glutamine Conc.	Medium Exchange Frequency	Mark As
1	3.5 g/L	2 mM	Once per day	3.5/2

2	5.0 g/L	4 mM	Once per day	5/4
3	7.0 g/L	6 mM	Once per two days	7/6
4	5.0 g/L	4 mM	¼ volume exchange per day	Spinner

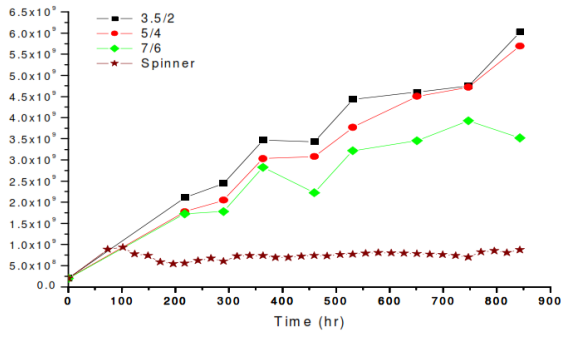
Result

Glucose uptake rate (mg/hr)



All culture reaches a stable GUR at around 250 to 300 hours. 5/4 unit has the highest GUR, and spinner flask is the lowest one due to the lower cell density. Note that even 5/4 got the highest GUR, but the cell density and productivity is very similar to that in the 3.5/2 unit (see data below). It hints that higher glucose concentration may not contribute positive effect on cell growth and protein production.

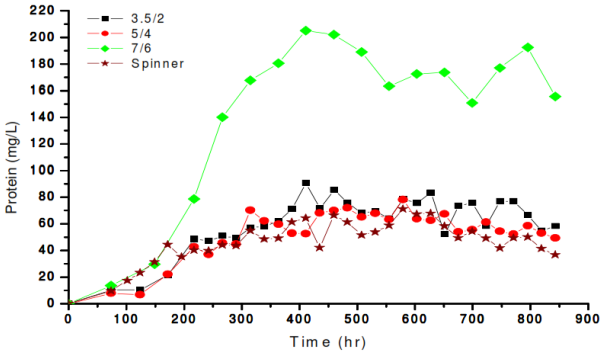
Cell growth curve



At the end of culture (843 hours), the cell number in each unit is:

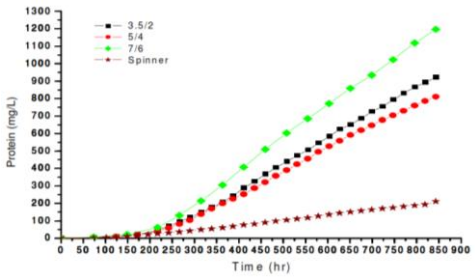
Unit	3.5/2	5/4	7/6	Spinner
Cell Number	6.02×10^9	5.69×10^9	3.52×10^9	6.2×10^8

IgG Production



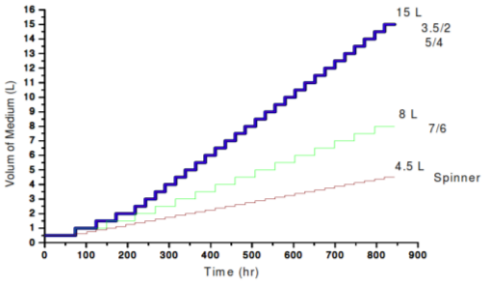
The IgG concentration in each unit keep rising until 400 hours. The highest concentration is 205.26 mg/L at the 7/6 unit. Other three units got very similar IgG concentration around 80 mg/L. The reason of the higher IgG concentration in the 7/6 unit is because it exchanges medium once per two days while still providing sufficient glucose and glutamine. It is interested to found that there is no negative effect in the low medium exchange frequency unit, i.e. 7/6 unit. Besides, it is the most productive one in the experiment as show in the below figure.

Accumulated IgG

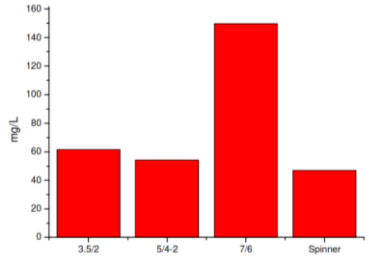


The accumulated IgG produced from different culture condition showing that the 7/6 one can produce more protein and save more culture medium. 1197 mg IgG protein are collected within 35 days culture in the 7/6 unit, while in spinner flask, only 210 mg IgG was produced within the same culture period. It is 5.7 folds increase compared with traditional 1 L spinner flask system.

Consumed Volume



The one in Spinner flask consumed less culture medium due to the 1/4 medium exchange strategy. While 3.5/2 and 5/4 consume most culture medium. In 7/6, it consumed 1.8 folds culture medium while produce 5.7 folds protein compared with spinner flask. The protein concentration in 7/6 is 3.2 folds higher than that in spinner flask.



The figure shows that the average protein concentration (mg/L) in the four groups. 3.5/2 (61.47 mg/L) and 5/4 (54.07 mg/L) is slightly higher than spinner flask (46.88 mg/L); while 7/6 is 149.63 mg/L, which is 3.2 folds concentrated than spinner. Showing the strategy of increase glucose and glutamine in culture medium and exchange once per two days can enhance protein production in this case.

Summary (7/6 unit only)

Seed	**Inoculum Volume**	**Medium Volume**	**Medium**
2 x 10^8 cells/bottle	50 ml	500 ml	HyQ-PF-CHO
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Final Cell Density (Nuclei Count)**
35 days	8L	15 times/ 35 days	3.5 x 10^9 cells/bottle
Max. GUR	**Total Protein Yield**	**Average Protein Concentration**	**Multiplication of Cells**
58.3 mg/hr	1197 mg	149.63 mg/L	17.6 fold

Please contact VacciXcell technical support for any questions or comments.

mail@vaccixcell.com

Website: http://vaccixcell.com/