CelCradle-500 Technical Report XV

CHO-β-Gal cell culture for β-gal protein production

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1 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, applications of CelCradle-500 for the growth of CHO-β-Gal cells and the production of β-gal with different medium conditions were illustrated. 5×107 CHO cells were seeded in each CelCradle-500 unit. A final cell population about 1.7×10^9 - 3.5×10^9 in one CelCradle -500 unit was obtained. For the protein production, β-gal total yield was equivalent to the production from 5440-14200 units. High cell density causes drastic pH change in culture medium. This study shows that culture with low glucose concentration, high quantity of cells and bioproducts were obtained in CelCradle-500 with stable pH controlled. This technical sheet provides a recommended protocol for users to start up their culture. However, the optimum condition of each cell culture for each case may require users to determine.

2 Material

Device	Cell Line/Product	Medium	Seed
CelCradle-500	CHOβ-Gal/β-gal	DMEM/F12, 5% FCS, 3.7 g/L $NaHCO_3$, 3 g/L glucose, 4 mM Glm	5 x 10^7 cells/bottle
CelCradle-500	CHOβ-Gal/β-gal	DMEM/F12, 5% FCS, 3.7 g/L $NaHCO_3$, 5 g/L glucose, 3.5 mM Glm	5 x 10^7 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare CHO-β-Gal cells by subculture in T-150 flasks or Roller bottles. After cell density reaches adequate number and cell viability remains above 95%, it is ready for the preparation of inoculation. Collect 1.0×10^8 cells from the culture flasks by trypsinization and separate into two 50 ml centrifuge tubes with each 30 ml fresh media.

3.2 Preparation before cell seeding

Place two CelCradle Stage controllers in two 37℃ incubators. Set up the inoculation parameters (See below). Warm up culture media in 37℃ water bath. Take out two CelCradle-500 bottles aseptically and

place it in a biosafety cabinet. Open the cap and add each bottle with 470 ml fresh culture medium in the bottle.

3.3 Inoculation

Open the cap and distribute 30 ml media containing 5.0 x 10^7 suspended cells 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.

3.4 Culture

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

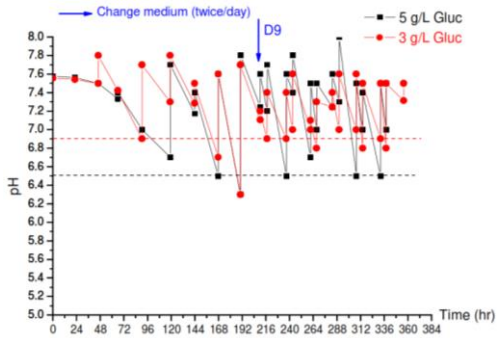
Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	0 sec	1.5 mm/s	1 min 30 sec

During culture, monitor the pH, residual glucose concentration and other metabolic in order to predict the time for medium replenishment. Below are the tips:

1. Adjusting down CO_2 concentration in CO_2 incubator until zero.
2. Adjust pH by adding 7.5% $NaHCO_3$.
3. Exchange culture medium with higher $NaHCO_3$ concentration.

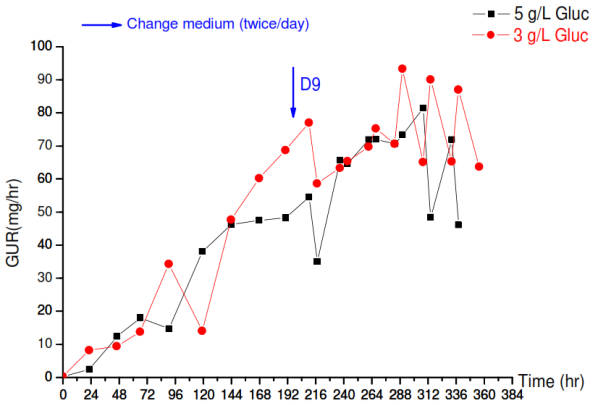
4 Result

pH

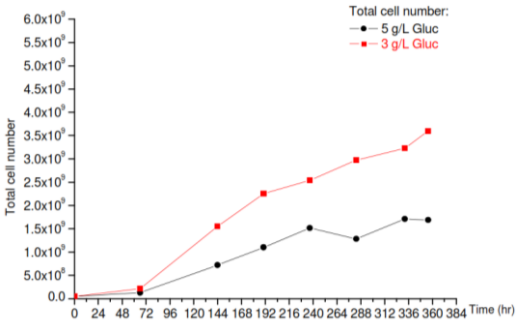


The pH value of low glucose medium (3 g/L Gluc) was maintained around 6.9 without turning off CO_2 in 37℃ incubator. However, even reducing CO_2 concentration, the pH value of high glucose medium (5 g/L Gluc) dropped to 6.5. Therefore, low glucose medium could provide a stable pH environment for CHO-β-Gal cells in CelCradle-500 during culture.

Glucose uptake rate (mg/hr)

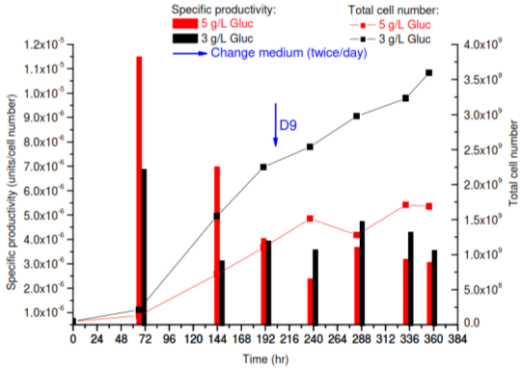


Cell growth curve



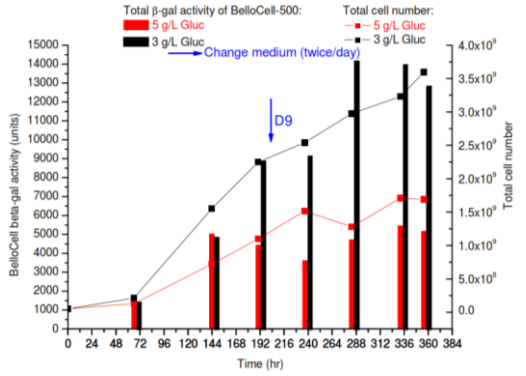
At the end of culture (362.3 hours), the total cell number in each bottle was: 5 g/L Gluc: 1.7×10^9, 3 g/L Gluc: 3.6×10^9. Final total cell number of low glucose condition was double of high glucose condition's. This result suggested that pH value was important for CHOβ-Gal cell growth and could be stably controlled around 6.9 by low glucose medium.

Specific productivity of β-gal



In high glucose's condition, the specific productivity of β-gal was declined in pace with cell growth. Differently, in low glucose's condition, the specific productivity was reduced at the beginning and then maintained at constant level during culture. In addition, before day 9 of culture, the specific productivity of high glucose's condition was higher than low glucose's. When we exchanged medium frequently up to twice per day, the specific productivity of low glucose's condition became higher than high glucose's. Based on this result, we derived that both the pH value and nutrient of the medium are important for CHOβ-Gal cells to produce β-gal.

Total β-gal activity of CelCradle-500



The maximal β-gal activity produced by one CelCradle-500 in low glucose's condition was at day 12 of culture. The amount was 14,200 units. In high glucose's condition, the maximal β-gal activity of CelCradle-500 was at day 14 and the amount was only 5440 units.

5 Summary

CelCradle-500 provides a powerful cell culture tool to achieve high cell density and high productivity of target bioproducts. However, high cell density will cause the pH value of the medium became acidic very fast. In the past, we adjusted CO2 concentration in CO2 incubator and/or increased NaHCO3 concentration in the fresh medium to control the pH value. In this application note, we demonstrated that large amount of CHOβ-Gal cells and β-gal could be obtained by culture with low glucose concentration. By this strategy, no adjustment is needed. This provides a simplified method for users to start up their culture if stable pH is required.

Seed	**Inoculum Volume**	**Medium Volume**	**Medium**
5 x 10^7 cells/bottle	30 ml	500 ml	HyQ-PF-CHO
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Total Cell Counted**
15 days	9500 ml	18 times	3.5 x 10^9 cells/bottle
Max. GUR	**Max. Protein Produced**	**Max. Total Cell Number**	**Multiplication of Cells**
93.33 mg/hr	14,200 units	3.5 x 10^9 cells/total	70 fold

Note:

1. CHO-β-Gal cell line is obtained from Culture Collection and Research Center (CCRC) in Taiwan. CCRC No. is CCRC 60377. Reference: In Vitro Cell Dev. Biol.-Animal 37: 633-634.
2. Protocol for β-gal assay:
   (1) Use forceps to pick out two carriers from carrier box of CelCradle-500.
   (2) Place two carriers in one 2 ml microcentrifuge tube.
   (3) Add 2 ml PBS into the tube and gently mix by inversion.
   (4) Remove PBS
   (5) Add 2 ml lysis buffer into the tube (2 carriers/2 ml lysis buffer)
   (6) Vortex the tube for 1 minute and then stand at 4℃ for 10 minutes; repeat this step for six times.
   (7) Spin the cell debris at 12,000×g for 5 minutes.
   (8) Transfer the supernatant into a fresh microcentrifuge tube and proceed to the β-gal assay.
   (9) Pipet 30 µl of cell lysate into a 96-well microtiter dish.
   (10) Add 130 µl buffer A-β-mercaptoethanol mixture to each well.
   (11) Cover and incubate the microtiter dish for 5 minutes at 37℃.
   (12) Add 50 µl of ONPG substrate to each well and cover the dish with a dish lid.
   (13) Incubate the dish at 37℃ until mixture turns bright yellow.

(14)Terminate the reaction by adding 90µl of stop solution and read at 420 nm.

3. β-gal assay is detected by β-galactosidase assay kit (STRATAGENE, Catalog #200383)
4. Units of β-gal is calculated by: OD_{420}/0.0045 = nmoles formed per milliliter

   nmoles/ml × total assay volume= nmoles

   nmoles/ time of 37℃ incubation= nmoles/minute (units)

6 VacciXcell Technical Support

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