CelCradle-500 Technical Report XII

Cultivation of Huh 7 Cells

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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 application of CelCradle-500 for growth of Huh-7 cells is illustrated.

1.1×10^8 cells/bottle was seeded and obtained a total of 2.43×10^9 cells counted by crystal violet dye nucleus count method at 309 hours, with a total 22 folds increase of cell population. Glucose concentration in the culture medium was monitored and kept above 1.0 g/L. 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	Medium	Seed
CelCradle-500	Huh-7	DMEM/10%FBS + 2.5 g/L glucose + 2.5 mM glutamine + 3.7 g $NaHCO_3$	1.1 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one roller bottle. Seed with 2.5 x 10^7 cells total. Culture at 37℃, 5% CO2 for total 5 days. Replenish medium at day 3. Harvest cells by standard trypsinization protocol. Prepare 1.1 × 108 suspended cells with viability above 95%, and concentrate cells in 50 ml culture medium.

3.2 Inoculation

Pre-warm DMEM/10%FBS medium in 37℃ water bath. Take out one CelCradle-500 bottle aseptically and place in a biosafety cabinet. Open the cap and add 450 ml culture medium in the bottle. Well distribute 30 ml prepared inoculums on top of the matrix box and bring to CelCradle Stage immediately. Fix the bottles on CelCradle Stage controller in CO_2 incubator with 37℃, and 5% CO_2 and start the run immediately.

3.3 Immobilization

Set up operation parameters on the CelCradle Stage control box and start the controller by pressing "START" button. 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

3.4 Culture

After 3.5 hours, switch the parameters to culture parameters. The culture control parameters are set as below:

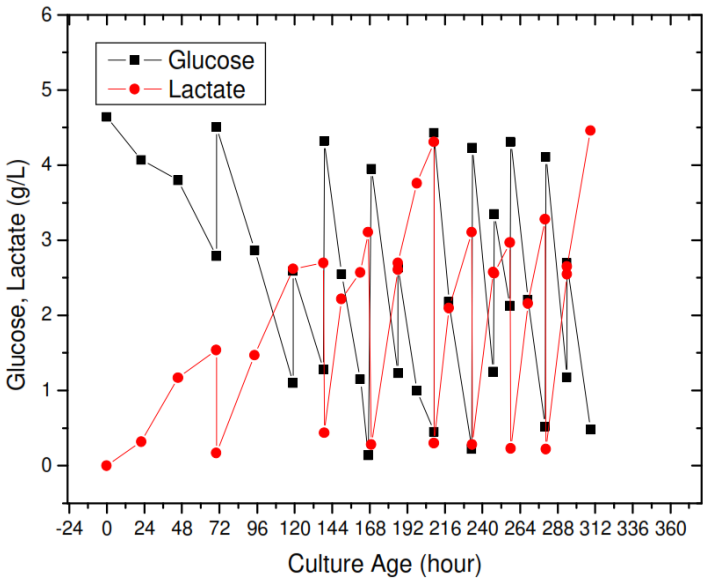
Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	0 sec	1.5 mm/s	1 min 30 sec

Monitor the residual glucose concentration and the color of medium in order to predict the time to change culture medium.

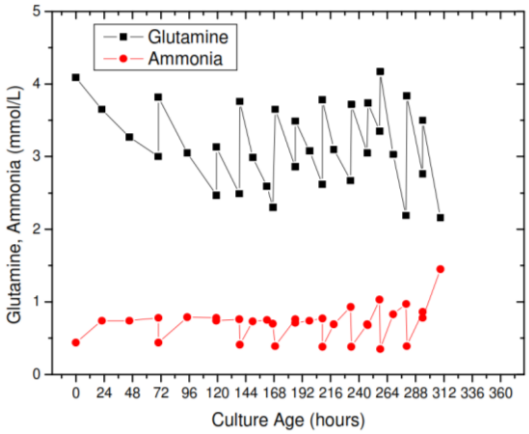
The setup parameters are only for reference. These are not necessarily the optimum parameter conditions.

4 Result

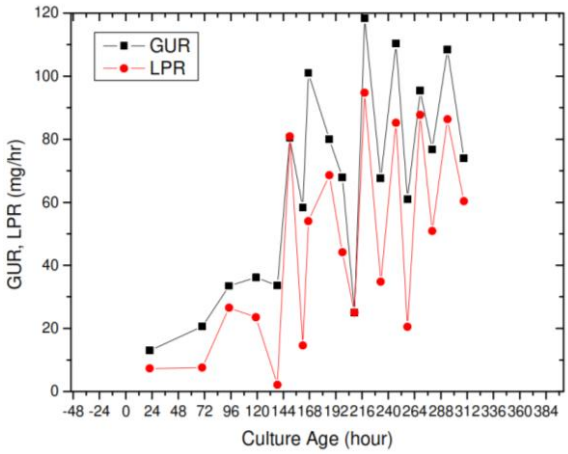
Glucose and Lactate profile



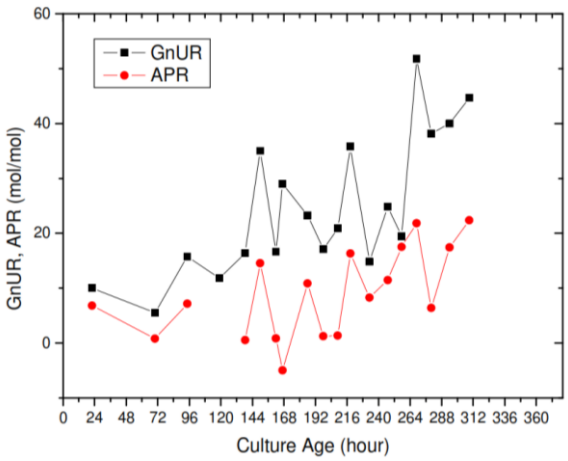
Glutamine and Ammonia profile



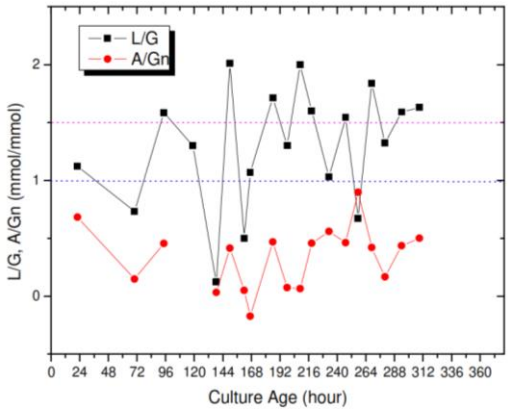
Glucose uptake rate (GUR) and Lactate production rate (LPR) profile



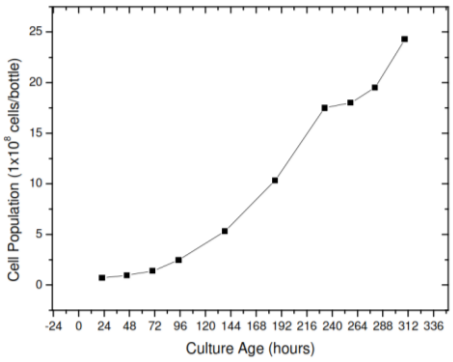
Glutamine uptake rate (GnUR) and Ammonia production rate (APR) profile



L/G and A/Gn

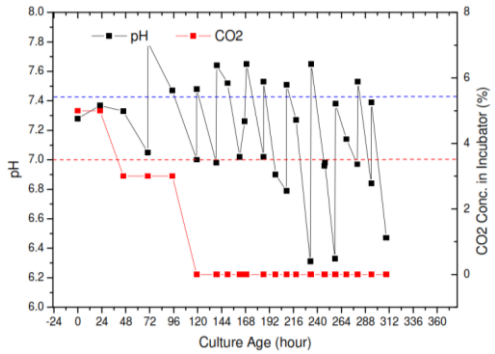


Cell grow curve by crystal violet dye nuclear count method



Cell propagation is exponentially increased until 240 hours. There is a little inhibition of cell growth after 240 hours and may due to the low pH within that period.

pH/CO_2



pH is below normal range after 240 hours culture. Cell growth is inhibited. We will suggest reducing initial glucose concentration to below 3.0 g/L and gradually increasing $NaHCO_3$ toabove 4.5 g/L and also adjust pH twice a day if the low pH situation occurred.

The growth of Huh-7 cells in CelCradle-500 is slower compared with other cell lines, it took 10 days to reach nearly 10 folds increase of cell population, and reach 2.43 × 10^9 cells/bottle. Cell doubling time is around 45.36 hr. GUR is very high and produce a lot of lactate. PH is out of control during late phase of culture and results an inhibition of cell growth. We suggest to reduce the initial glucose concentration to below 3 g/L and gradually increase $NaHCO_3$ to above 4.5 g/L in order to control the pH within range.

5 Summary

Seed	**Inoculum Volume**	**Medium Volume**	**Medium**
1.1 x 10^8 cells/bottle	50 ml	500 ml	DMEM/10%FCS
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Final Cell Density (Nuclei Count)**
309 hours	2850 ml	5 times	2.43 x 10^9 cells/bottle

6 VacciXcell Technical Support

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