CelCradle-500 Technical Report XI

Cultivation of QBI-HEK 293 Cells

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1 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 CelCradle-500 for the growth of QBI-HEK-293A cells is illustrated. 1.07 x 10^8 cells/bottle and 2.68 x 10^8 cells/bottle was seeded to verify the seeding density effect in the CelCradle-500 system. A total of 2.58 x 10^9 cells and 2.94 x 10^9 cells counted by crystal violet dye nuclear count method at 216 hours, with a total 24 and 11 folds increase of cell population. Higher seeding density did not promote cell growth in this study. 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	QBI-HEK-293A (QBIGENE)	DMEM/10% FBS + 3.7 g $NaHCO_3$	1.07 x 10^8 cells/bottle 2.68 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare 3 roller bottles. Seed with 2.5 x 10^7 cells in each bottle. Culture at 37°C, 5% CO_2 for a total of 5 days. Replenish medium at day 3. Harvest cells by standard trypsinization protocol. Prepare 1.0×10^8 and 2.0×10^8 cells/bottle suspend 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 BelloCell-500 bottle aseptically and place in a biosafety cabinet. Open the cap and add 450 ml culture medium in each bottle. Dispense 50 ml prepared inoculums on top of the matrix box and bring to BelloStage immediately. Fix the bottles on BelloStage 3000 controller in CO_2 incubator with 37°C and 5% CO_2 and start the run immediately.

3.3 Immobilization

Set up operation parameters on the BelloStage 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 2.5 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

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

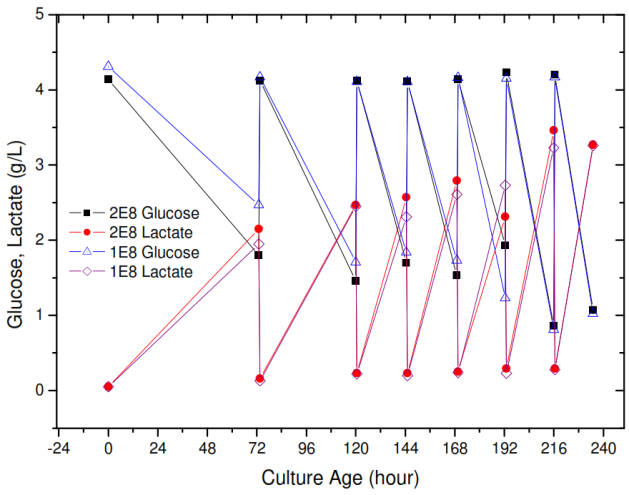
The setup parameters are for reference only. These are not necessarily the optimum parameter conditions.

3.5 Cell Count

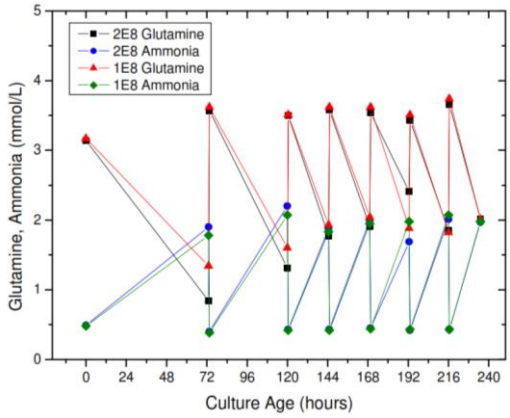
Cell count was done by crystal violet dye nucleus count method according to the protocol on user manual CD.

4 Result

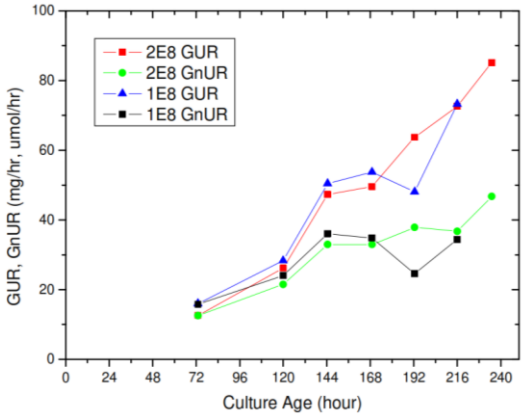
Glucose and Lactate profile



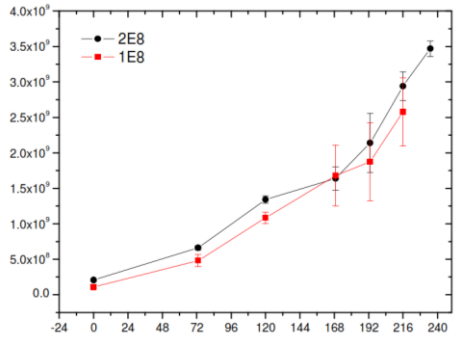
Glutamine and Ammonia profile



Glucose uptake rate (GUR) and Glutamine uptake rate (GnUR) profile

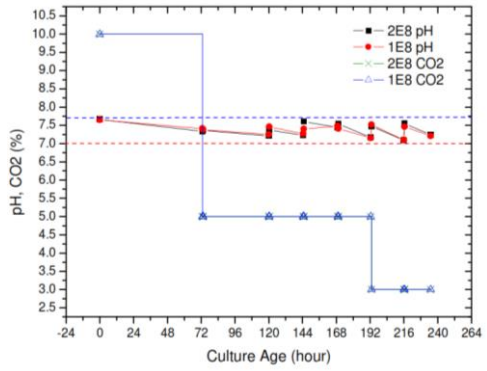


Cell growth curve by crystal violet dye nuclear count method



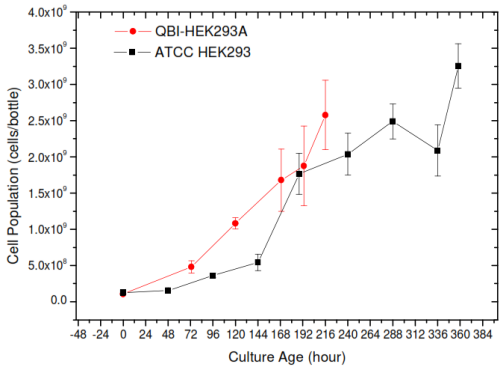
Growth profile between two different seeding densities is similar. It demonstrates that 1×10^8 cells is enough for a normal growth for the QBI-HEK293A cell line. Higher seeding density did not benefit the growth or shorten the culture period.

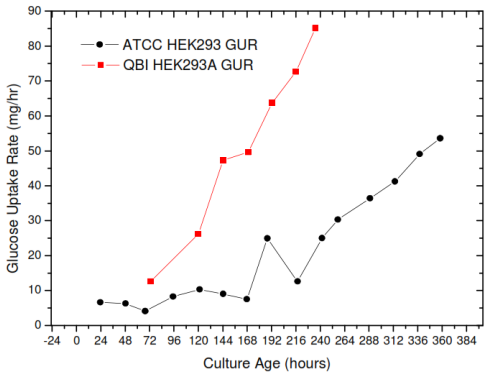
pH/CO_2



CO_2 concentration still keeps above 3% during the whole culture process while can maintain pH within normal range. Both HEK293 cell lines from both QBIGENE and ATCC shows relatively stable pH during culture compared with other cell lines such as CHO.

Comparison between HEK293 from ATCC and QBIGENE





There is basic growth difference between the two sources of HEK-293. The one from ATCC generates a longer lag phase in our system, while the one from QBIGENE did not show any sign of lag phase. The one from ATCC has a five days lag phase showing in glucose consumption rate. The major suspect for the long lag phase is due to the poor immobilization efficiency on HEK293, which is sensitive to trypsin. We suggest customers to use BelloCell-500AP for those cells with poor immobilization

efficiency. Therefore, the cell growth with QBI-HEK293A cells shows a relative normal growth profile than the one from ATCC due to there are more QBI-HEK293A cells adhered on matrix than that from ATCC. The culture of QBI-HEK 293A cells in BelloCell-500 is very smooth, pH is relatively stable compared with other cell line. It took 10 days in BelloCell-500 system for HEK293 cells to reach around 3.5×10^9 cells/bottle.

5 Summary

Seed	**Inoculum Volume**	**Medium Volume**	**Medium**
1.07/2.68 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)**
240 hours	3500 ml	6 times	3.5 x 10^9 cells/bottle

6 VacciXcell Technical Support

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