

# CelCradle-500 Technical Report VIII

## Cultivation of HEK 293 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 HEK-293 cells is illustrated.  $1.27 \times 10^8$  cells/bottle was seeded and obtain a total of  $3.26 \times 10^9$  cells counted by crystal violet dye nuclei count method at 358 hours, with a total 26 folds increase of cell population. It took 7 days to grow from  $1.27 \times 10^8$  cells to  $2 \times 10^9$  cells. However, it took another 7 days to grow from  $2 \times 10^9$  cells to  $3 \times 10^9$  cells. 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	HEK-293	$\alpha$ MEM/10%FBS + 2.5g/L glucose + 2.5 mM glutamine + 2.2 g NaHCO <sub>3</sub>	$1.27 \times 10^8$ cells/bottle

## 3 Protocol

### 3.1 Inoculum Preparation

Prepare one roller bottle. Seed with  $2.5 \times 10^7$  cells total. Culture at 37°C, 5% CO<sub>2</sub> for total 5 days. Replenish medium at day 3. Harvest cells by standard trypsinization protocol. Prepare  $1.27 \times 10^8$  suspend cells with viability of 97.42%, and concentrate cells in 50 ml culture medium.

### 3.2 Preparation before cell seeding

Place CelCradle controller in a 28°C incubator. Set up the inoculation parameters (See below). Warm up SF900 II medium in 28°C water bath. 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

Pre-warm  $\alpha$ MEM/10%FBS medium in 37°C 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 each bottle. Dispense 50 ml media containing  $1.27 \times 10^8$  suspend cells on top of the matrix of CelCradle-500. Bring the bottle and lock up on the CelCradle console in incubator at 37°C, 5% CO<sub>2</sub> and start the run immediately. Avoid swirling or shaking the bottle before start compression.

### 3.4 Immobilization

Set up operation parameters on the CelCradle 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

### 3.5 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.

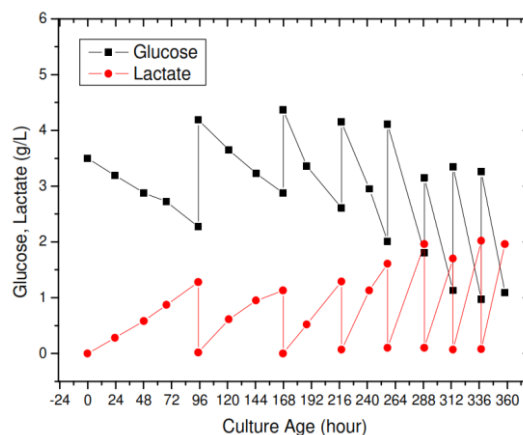
*The setup parameters are only for reference. It does not necessary to be optimum parameters.*

### 3.6 Cell Harvest

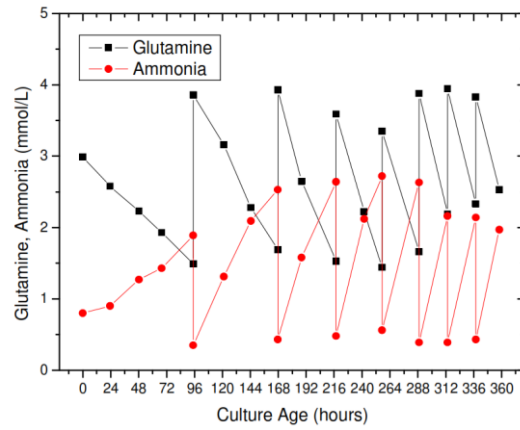
Monitor the residual glucose concentration and the color of medium in order to predict the time to change culture medium.

## 4 Result

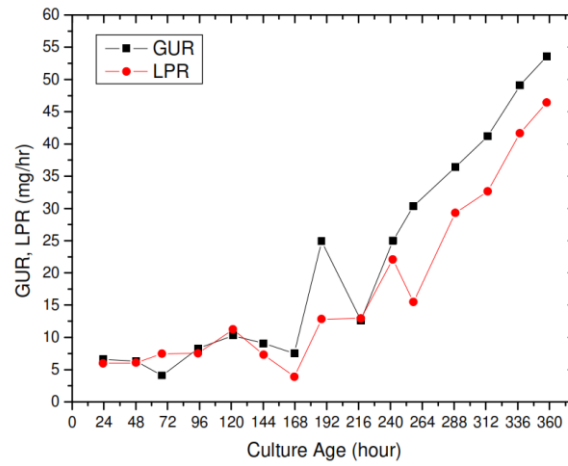
### Glucose and Lactate Profile



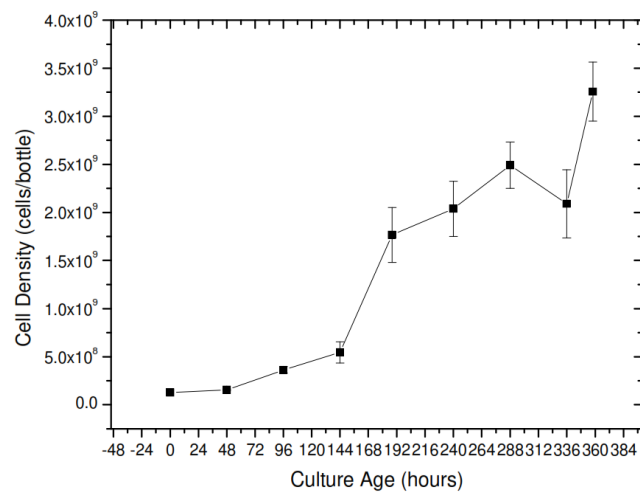
Glutamine and Ammonia Profile



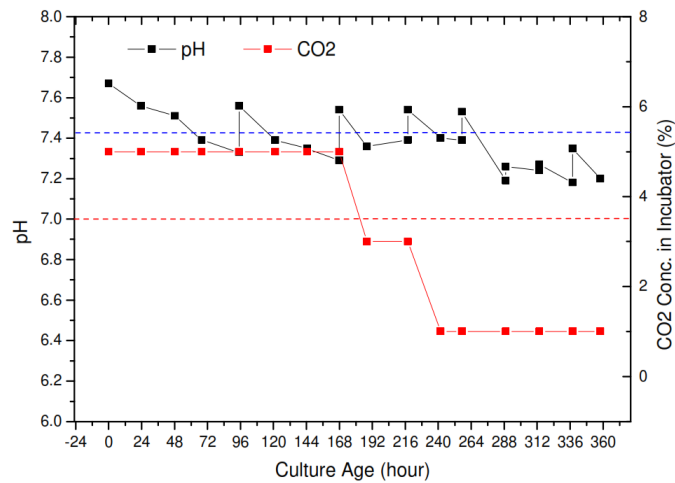
Glucose uptake rate and Lactate production rate profile



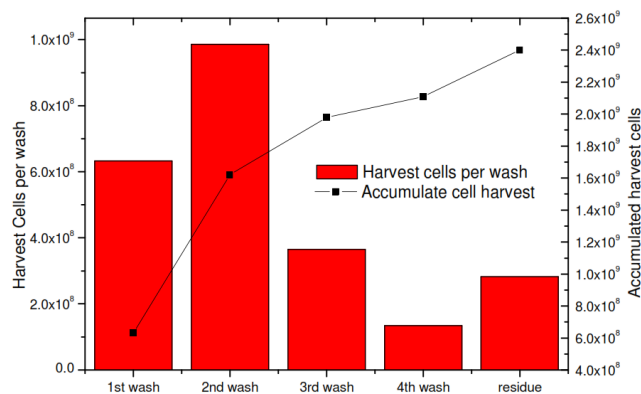
Cell grow curve by crystal violet dye nuclear count method



pH/CO<sub>2</sub>



Cell Harvest



The culture of HEK 293 cells in CelCradle-500 is very smooth and the grow rate is slower than the other commonly used cell line, it require 7 days to have nearly 20 folds increase of cell population. The maximum cell density in CelCradle-500 system for HEK293 cells is around 3.5 x 10<sup>9</sup> cells/bottle, and will require 12 days culture to reach this value. For adenovirus production, we suggest to seed cells with 2x10<sup>8</sup> cells/bottle, and culture for 6-7 days until cell density reach above 2x10<sup>9</sup> cells before start of infection.

**Note**

HEK293 cell is very sensitive to trypsin and easy to detach. Over trypsinizing the cells will cause the difficulty for cells to be immobilized in the bottle and cause a result of slow growth or even fail to growth. To avoid this, try to minimize cell dissociation process by shortening the trypsinization incubation time (within 3 mins) and terminate the enzymatic reaction by adding serum or trypsin inhibitor. CESCO also

develops another bottle to enhance cell immobilization efficiency, i.e. CelCradle-500AP. If users are interested with the product, please contact VacciXcell directly.

## 5 Summary

<b>Seed</b>	<b>Inoculum Volume</b>	<b>Medium Volume</b>	<b>Medium</b>
1.27 x 10 <sup>8</sup> cells/bottle	50 ml/bottle	500 ml/bottle	αMEM/10%FCS
<b>Total Culture Age</b>	<b>Total Medium Consumed</b>	<b>Total Medium Replenish Frequency</b>	<b>Final Cell Density</b>
356 hours	3500 ml	6	3.25 x 10 <sup>9</sup> cells/bottle

## 6 VacciXcell Technical Support

For queries and comments, please contact the VacciXcell Technical Support team.

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