CelCradle-500P Technical Report II

Cultivation of MDCK cells

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1 Description

CelCradle-500P 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 non-secreted products from one CelCradle-500P bottle. In this study, the application of CelCradle-500P for growth of MDCK cells is illustrated. The following experiments were performed by culturing MDCK cells (Canine Kidney cells, ATCC CCL-34) in CelCradle-500P for 168 hours. Final 1.2 x 10^9 cells cells were achieved in one CelCradle-500P bottle. The cell line is a strong contact-inhibition cell line and doesn't form 3-D tissue structure as other cell lines and that's the major reason to achieve lower cell density than the other cell lines. 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-500P	MDCK ATCC CCL-34	Plus MDCK (VacciXcell)	5.7x10^7 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one roller bottle with sub-confluence cells (confluence with make cell harvest difficult). Harvest cells by standard trypsinization protocol. Prepare 5.0 x 10^7 to 1.0 x 10^8 suspended cells and concentrate cells in 50 ml culture medium.

3.2 Preparation before cell seeding

Place CelCradle Stage controller in a 28℃ incubator. Set up the inoculation parameters (See below). Warm up SF900 II medium in 28℃ 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 Plus MDCK medium in 37℃ water bath. Take out one CelCradle-500P bottle aseptically and place in a biosafety cabinet. Open the cap and add 450 ml culture medium in each bottle. Dispense 50 ml prepared inoculum on top of the matrix of CelCradle-500P. Bring the bottle and lock up on the CelCradle Stage console in incubator at 37oC, 5% CO2 and start the run immediately.

3.4 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

3.5 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 for reference only. These are not necessarily the optimum parameter conditions.

3.6 Cell Harvest

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

3.7 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

3.8 Culture

After 3 hours, switch the parameters to culture parameters. The culture control parameters are set as below:

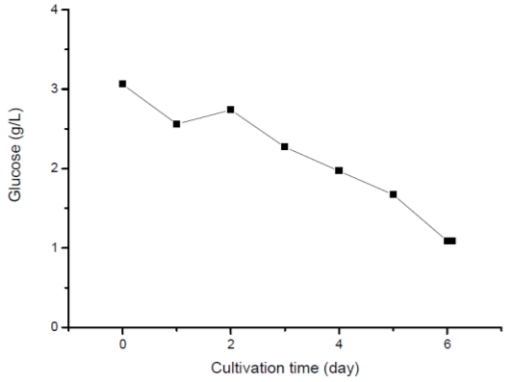
Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.0 mm/s	10 sec	1.0 mm/s	30 sec

3.9 Recirculation

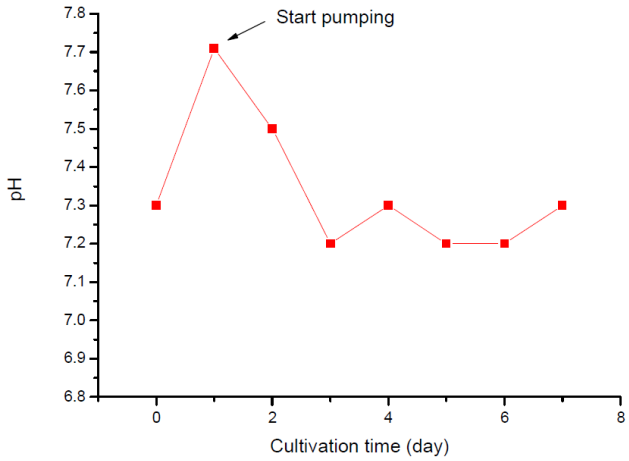
Connect the bottle with reservoir containing 2.2 L culture medium 24 hours after seeding then start recirculation. Start with 1000 mL/day with 24 cycle/day and increase to 1999 mL/day when the pH drops below 7.0. Sequentially adjust the CO_2 concentration in the incubator as the pH of medium decreases. Cell growth will reach to plateau in around 7 days.

4 Result

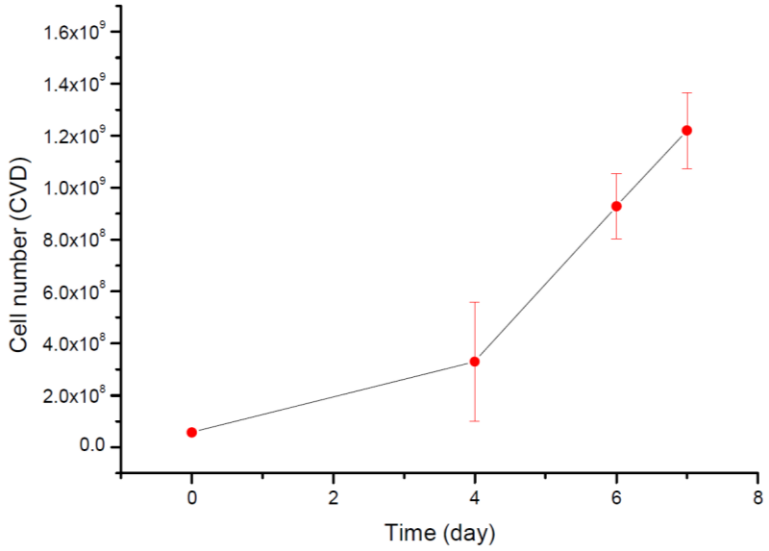
Glucose Concentration Profile



pH



Cell grow curve by crystal violet dye nuclear count method



The result shows that MDCK is a kind of strong contact-inhibition cell line that is difficult to form 3D tissue structure in CelCradle. Therefore, the overall cell density in CelCradle will be limited. When cell density reaches 1.2 × 10^9 cells, extra cells start to dislodge from the matrix and overall cell density will keep the same. Therefore, it is suggest to seed 5 x 10^7 cells and the culture should be able to reach plateau at around 6 days culture.

5 Summary

Seed	Inoculum Volume	Medium Volume	Medium
5.7 x 10^7 cells/bottle	50 ml/bottle	500 ml/bottle	Plus MDCK (VacciXcell)
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Final Cell Density**
7 days	2700 ml/bottle	0	1.2 x 10^9 cells/bottle

6 VacciXcell Technical Support

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