CelCradle-500AP Technical Report II

Cultivation of MDCK cells

Table of Contents

1 Description

CelCradle-500AP 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. In this study, the application of CelCradle-500AP for growth of MDCK cells is illustrated. The following experiments were performed by culturing MDCK cells (Canine Kidney cells, ATCC CCL-34) in CelCradle-500AP for 168 hours. Final 1.2 x 10^9 cells were achieved in one CelCradle-500AP bottle. The cell line is a strong contact-inhibition cell line and does not form 3-D tissue structure as other cell lines do and it is the major reason resulted in lower cell density compare to other cell lines. In addition, this technical sheet provides a general protocol for users to start up the culture experiment. The optimum condition of each cell culture for each case will vary depends on individual's requirement.

2 Material

Device	Cell Line	Medium	Seed
CelCradle-500AP	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 5.0×10^7 to 1.0 x 10^8 suspended cells and concentrate cells in 50 ml culture medium.

3.2 Inoculation

Pre-warm Plus MDCK medium in 37℃ water bath. Take out one CelCradle-500AP 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-500AP. Bring the bottle and lock up on the CelCradle Stage console in incubator at 37°C, 5% CO_2 and start the run immediately. Avoid swirling or shaking the bottle before start compression.

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 hours, reset the parameters to culture parameters as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.0 mm/s	2 mins	1.0 mm/s	30 sec

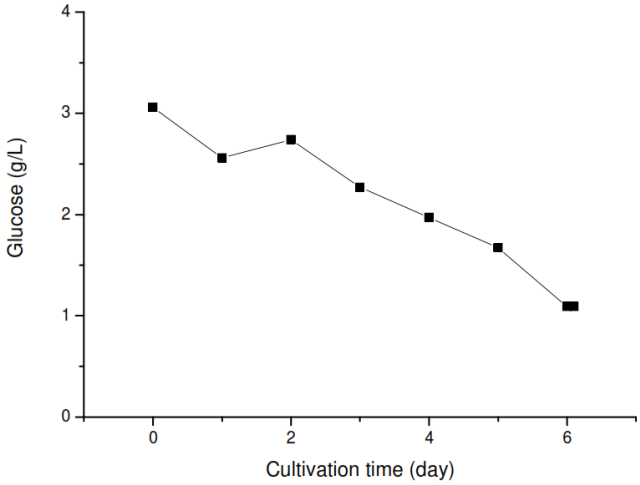
The setup parameters are only for reference. It does not necessary to be optimum parameters. VacciXcell will update recent data on the website, please check the website for updated information.

3.5 Recirculation

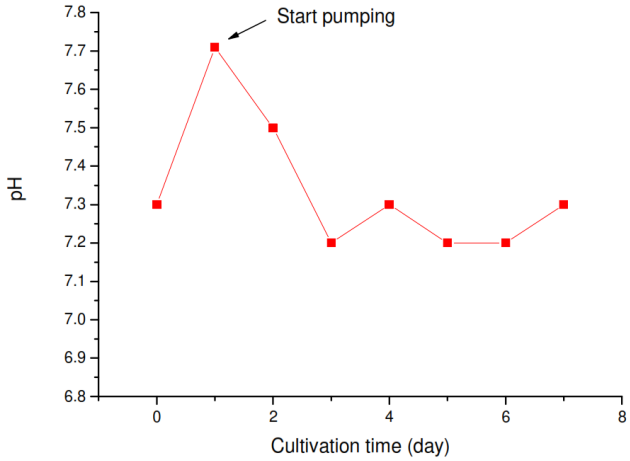
Connect the bottle with reservoir containing 2.2 L culture medium 24 hours after seeding then start recirculation. Start with 1000 mL/day with 24 cycle/day and increase to 1999 mL/day when the pH drops below 7.0. Sequentially adjust the CO_2 concentration in the incubator as the pH of medium decreases.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

4 Result

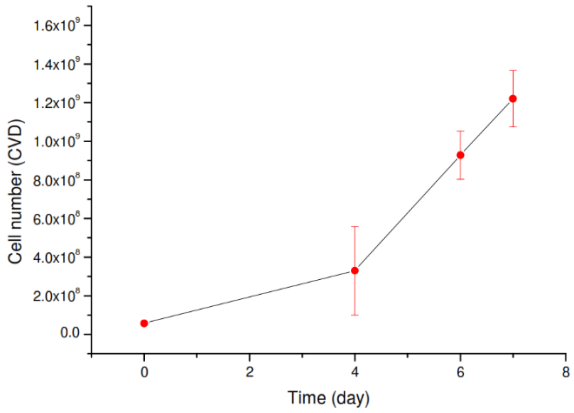
Glucose Concentration Profile



pH



Cell Growth (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 was limited. When cell density reaches at the maximum of 1.2 x 10^9 cells, additional cells hardly attach onto the matrix. Therefore, it is suggested to seed 5 x 10^7 cells and the culture should be able to reach plateau in around 6 days of culture.

5 Summary

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

6 VacciXcell Technical Support

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

Email: mail@vaccixcell.com

Address: 21 Changi South Street 1, Singapore 486 777

Telephone: +65 6542 0833

Website: http://vaccixcell.com/