CelCradle-500AP Technical Report I

Seeding Efficiency Enhancement with CelCradle-500AP Bottle

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1 Description

CelCradle-500AP provides an alternative choice from regular CelCradle-500 bottles specially designed to enhance cell immobilization, virus infection, transit transfection or cell harvest efficiency, or to harvest carriers directly.

In this study, the validation of cell immobilizatioin efficiency with a CelCradle-500P bottle and a CelCradle-500AP bottle was illustrated by using an over trypsized HEK-293A cells. Of 88% immobilizatioin efficiency was achieved within 3 hours seeding period in CelCradle-500AP bottle; in contrast to 40% in CelCradle-500P bottle with regular seeding protocol. This study shows that the CelCradle-500AP bottle enhances the cell immobilization for slow adherent or over trypsinized cell lines such as HEK293 cells.

2 Material

Device	Cell Line/Product	Medium	Seed
CelCradle-500AP CelCradle-500P	HEK-293A (Trypsizined over 30 mins)	DMEM/10%FCS	1.0 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Inoculate HEK-293A cells in 6 x T150 flasks each with 3.0 x 10^6 cells in 30ml DMEM/10%FCS pre-warmed culture medium. Incubatore 37^{o}C 5% CO_2 incubator for 3 days. Over trypsinize cells in T150 flasks for 30 minutes treatment. Collect 2.0 x 10^8 HEK-293A cells by centrifugation and separate cells into 1.0 x 10^8 each in a 50 ml tube and a 200 ml bottle with 30 ml and 150 ml fresh culture medium. These cells were ready for CelCradle-500P, and CelCradle-500AP bottles inoculation. (Note: Cell viability should be above 90%)

3.2 Seed Experiment Design

Seed Protocol	Bottle	Initial Culture Medium (ml)	Inoculation volume (ml)	Seed (cells/ml)	Medium added after seeding (ml)
Regular protocol with continuous compression	CelCradle-500P	470	30	1 x 10^8	0
Reverse Bottle protocol	CelCradle-500AP	0	150	1 x 10^8	350

For seed method 1

Add 470 ml fresh culture medium in a CelCradle-500P bottle. Open the cap and dispense 30 ml media containing 1.0×108 suspended cells above the matrix box. Bring the bottle and lock up on the CelCradle Stage controller in incubator at 37°C immediately. Press “START” button to start the controller. The inoculation parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
2.0 mm/s	1 min	2.0 mm/s	0 sec

For Seed method 2

Dispense 150 ml culture meidum containing 1.0 × 10^8 suspended cells directly on the carriers in a CelCradle-500AP bottle. Cap with a non-vented cap. Reverse the bottle and allow the matrix and seed fall to the cap. Mix the seed with the matrix by swirling the bottle. Place the bottle in a CO_2 incubator. Sampling every 1 hour to check the seeding rate.

3.3 Culture

After 3-4 hours, reset the parameters to culture parameters as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	10 sec	1.5 mm/s	30 sec

Start recirculation by the second day (~24 hours) with pump rate 1 L/day, 24 cycle/day. Increase the pump rate to 1999 ml/day, 24 cycle/day by day 4.

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

4 Results

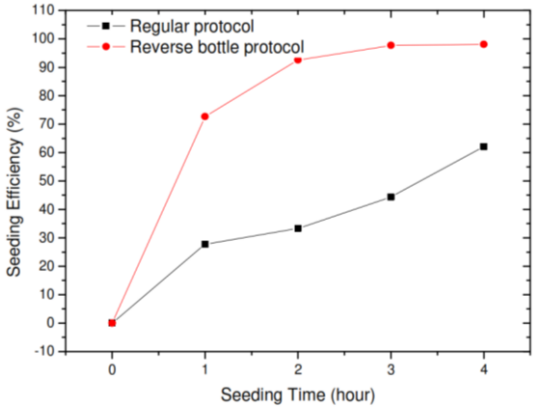

Fig.1 Comparison of cell immobilization efficiency between CelCradle-500P and CelCradle-500AP bottles with regular and reverse bottle seeding protocol.

By applying over-trypsinized HEK-293A cells to simulate cells with poor adherent capability, the CelCradle-500AP bottle with reverse bottle seeding protocol could enhance cell seeding efficiency. The result shows that seeding efficiency in reverse bottle protocol can reach 92.6% within 2 hours, and 98% in 4 hours. In contrast to regular seeding protocol, only 33.3% in 2 hours, and 62% in 4 hours. This clarifies some failure tests in CelCradle bottles with regular protocol especailly for cells with poor adherent capability. Therefore, for those cells with poor adherent capability, CelCradle-500AP is most favourable.

5 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/