CelCradle-500AP Technical Report IV

Antibody Production by Transient Transfection on HEK293T Cells

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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, transient transfection, cell harvest efficiency, or to harvest carriers directly.

In this study, a test of protein expression by transient transfection with CelCradle-500AP bottle was illustrated with HEK-293T cells. Two transient transfection reagents, namely linear PEI, and calcium phosphate ($CaPO_4$) were used and compared. Transfection was performed when cell density reaches $1x10^9$ cells/bottle. A yield of 35 mg antibody with linear PEI and 24 mg antibody with calcium phosphate was achieved. This study shows that the CelCradle-500AP bottle provides as an efficient tool for transient transfection at 10 cell density level.

2 Material

Device	Cell Line	Medium	Seed
CelCradle-500AP	HEK-293T	1/4 x DMEM/10%FCS 3/4 x VP-SFM (Gibco)	$1.0x10^8$ cells/bottle

Plasmid DNA	Transfection Reagent	Product
P3002-neo-HCMV20 (DCB, c-lambda)	Polyethyleneimine (PEI), Linear, MW 25,000 (Polysciences)	P3002-neo-HCMV20 Monoclonal Antibody

3 Protocol

3.1 Inoculum Preparation

HEK293T cells have been adapted in 1/4 x DMEM/10%FBS + 3/4 x VP-SFM. Prepare $1.0x10^8$ HEK-293T cells by centrifugation and resuspend cells in a 200 ml bottle with 150 ml fresh culture medium. These cells were ready for CelCradle-500AP bottles inoculation. (Note: Cell viability should be above 90%)

3.2 Inoculation

Dispense 150 ml culture medium containing $1.0x10^8$ 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 cells fall down on the cap. Mix the seed with the matrix by swirling the bottle. Place the bottle in a CO2 incubator with 37 C. Swirl the bottle every 30 mins for 3~4 hours.

3.3 Cell Culture

After 3~4 hours, up-right the bottle. Tap the bottle until all carriers fall back to the matrix basket. Exchange the non-vented cap with filtered cap. Add 350 ml culture medium. Lock the bottle in the CelCradle Stage. Setup the parameters in CelCradle Stage as below and start the run:

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

Monitor the residual glucose exchange culture medium by day 4^{th} and after glucose concentration below 1 g/L. 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.

3.4 Cell Transfection

Prepare to transfect 293T cells with linear PEI or CaPO4 when cell numbers reach to 1 X 10^9 cells/bt. Control group was done by adding transfect reagent without DNA.

For linear PEI

(1) Add 1.25 mg endotoxin free plasmid DNA in 50 ml 150 mM NaCl
(2) Add 2.5 ml linear PEI (stock, 1 mg/ml) in 50 ml 150 mM NaCl
(3) Mix (1)+(2) gently and stand at room temperature for 30 min
(4) Discard culture medium from the bottle. Drop-wise the mixture into cell culture, cap with a sterile non-vented cap, reverse the bottle, and incubate for 4 hours. Swirl the bottle to mix cells and transfection mixture gently every 30 min. Better performance will be able to achieve if place the reversed bottle in an orbital shaker with continuous shaking for 4 hours
(5) Add 400 ml culture medium into AP bottle, harvest cell culture at different days.

For $CaPO_4$

(1) Add 10 mg endo-toxin free plasmid DNA in 90 ml in DDW
(2) Add 10 ml 2.5M $CaCl_2$ solution into (1) DNA solution
(3) Drop-wise 100 ml 2X HBS (0.074 g KCl, 1.19 g HEPES, 0.2g dextrose, 1.6g NaCl, 0.0267 g $Na_2HPO_4 \cdot 2H_2O$) into (1) + (2) mixture. Do this while blowing bubbles
(4) Stand at room temperature for 30 min
(5) Discard culture medium from the bottle. Drop-wise the mixture into cell culture, cap with a sterile non-vented cap, reverse the bottle, and incubate for 4 hours. Swirl the bottle to mix cells and transfection mixture gently every 30 min. Better performance will be able to achieve if place the reversed bottle in an orbital shaker with continuous shaking for 4 hours;

(6) Add 300 ml culture medium into AP bottle, harvest cell culture at different days.

3.5 Harvest

Collect medium every four days until total volume reach 1.5 L.

4 Result

Cell Growth

For seeding with $1x10^8$ cells initially, it took 10 days to grow to $1x10^9$ cells. Higher seeding density with above $2x10^8$ cells would be suggested to reduce the culture days. Seed with $5x10^8$ reduced the culture day to 5 before transfection without requiring exchanging culture medium (data not shown).

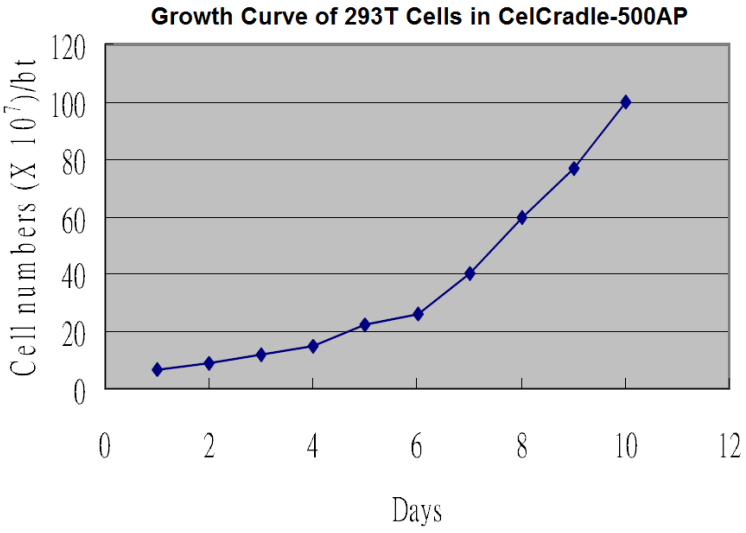

Figure 1. Cell growth culture of HEK293T

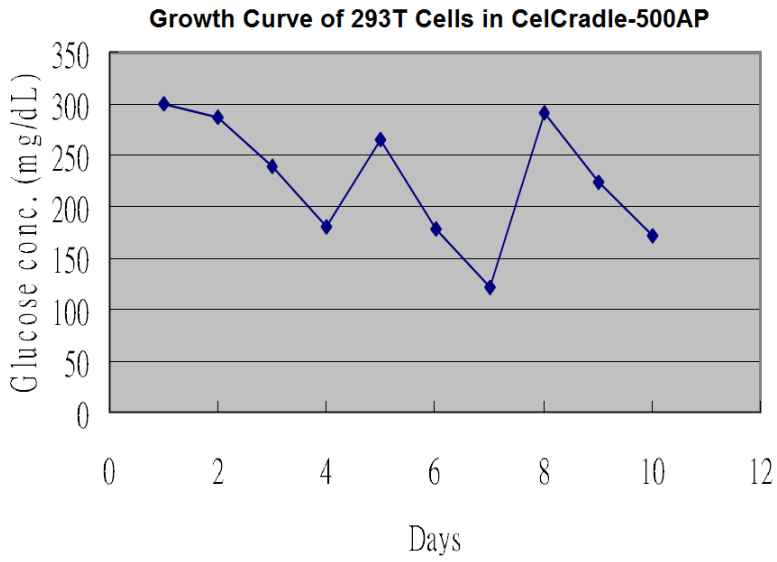

Figure 2. Glucose concentration profile during cell culture

Protein Expression

Table 1 shows the protein expression result with both linear PEI and $CaPO_4$. Overall yield is 35 mg antibody with linear PEI method and 24 mg antibody with $CaPO_4$ method, indicating a better transfection performance or lower cytotoxicity with linear PEI method.

Table 1. Antibody productivity with linear PEI and $CaPO_4$ transfection

Day	Day 3	Day 6	Day 9
$CaPO_4$	Harvest 500mL 12 µg /mL	Harvest 500mL 18 µg /mL	Harvest 500mL 18 µg /mL
Control of $CaPO_4$	Harvest 500mL 0 µg /mL	Harvest 500mL 0 µg /mL	Harvest 500mL 0 µg /mL
Linear PEI	Harvest 500mL 19 µg /mL	Harvest 500mL 25 µg /mL	Harvest 500mL 26 µg /mL
Control of Linear PEI	Harvest 500mL 0 µg /mL	Harvest 500mL 0 µg /mL	Harvest 500mL 0 µg /mL

Antibody Activity Confirm

The function of expressed antibody was further examined by a functional assay- ONPG assay. Result shows that the antibody has normal activity to inhibit HCMV virus to infect MRC-5 cells.

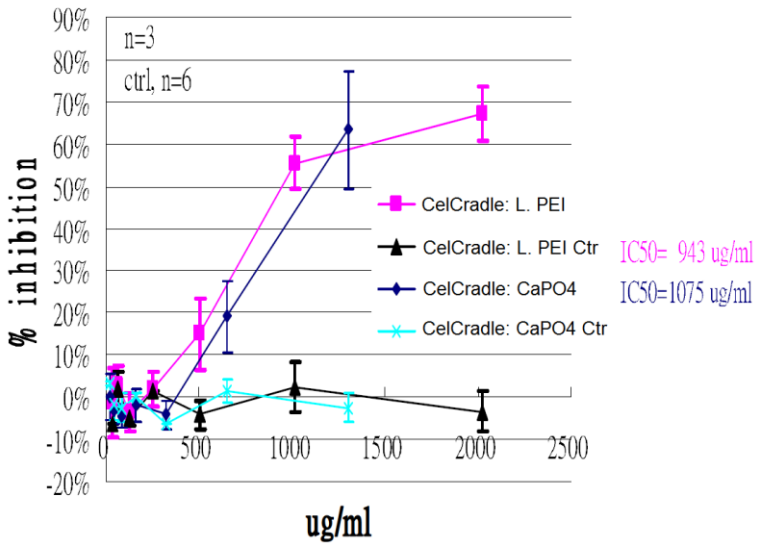

Figure. 3 Antibody activity assay result

The test of CelCradle-500AP for HEK293T cell growth and transient transfection indicates that CelCradle-500AP provides an efficient and alternative tool as the other cell culture devices such as tissue culture flasks, petri dishes, or roller bottles for transient transfection and protein expression for adherent cells. The special funtion provided by CelCradle-500AP by reversing the bottles mimicking the function as petri dishes for helping DNA complex to settle down on cell layers for efficient DNA uptake.

5 VacciXcell Technical Support

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