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PROTOCOL FOR GENERATION OF HEK293T SEED TRAIN USING CELCRADLE

Contents

Description

CelCradle™ proves to be a powerful tool to achieve high cell density in a cell culture because of its unique feature of offering high oxygen transfer and low shear stress culture environment. Users can easily collect highly concentrated cells as “seed” for larger scales of bioreactor like TideXcell-002 or to generate high-density cell banking. In this study, the application of CelCradle-500A and 500AP for the growth of HEK293T cells are illustrated. $0.75x10^8$ cells/bottle was seeded and a total of $2.44x10^9$ cells/bottle was obtained. To observe daily cell growth, live cells counting were performed via trypsin harvest. Results showed a total 33-fold increase within 8 days while maintaining its high metabolic rate. No significant difference in cell growth were observed between batch versus perfusion system. In addition, with less media change in perfusion system, cells experience less pH and glucose shock compared to batch system. This technical sheet provides a general protocol for users to start-up their culture. However, optimum condition of each cell culture for each case may vary.

Materials

- 0.01 % poly-L-lysine (Sigma P4707)
- PBS without Mg2+/Ca2+
- HEK293T (ATCC: CRL-3216)
- Complete media:
    - DMEM high glucose basal media (Hyclone SH30022.01)
    - 1X Pen/Strep (ThermoFisher Scientific 15141022)
    - 25 mM HEPES (Gibco 15630080)
    - 10% FBS (Hyclone SV30160.03HI)
- CelCradle™ Stage 3000
- CelCradle™ 500A (EscoAster/VaccixCell)
- CelCradle™ 500AP (EscoAster/VaccixCell)
- GlucCell Meter
- GlucCell Test Strips
- pH meter
- Long forceps
- Plastic sieve
- Florescent diacetate (FDA) (1 µg/ml) (ThermoFisher Scientific)
- Propidium Iodide (PI) (1 µg/ml) (ThermoFisher Scientific)
- Hoechst (1 µg/ml) (ThermoFisher Scientific)
- PrestoBlue Assay (ThermoFisher Scientific)
- 0.05 % Trypsin-EDTA (ThermoFisher Scientific 25300054)
- 75 g/L sodium bicarbonate (0.22 µm filtered)
- 100 g/L glucose solution (0.22 µm filtered)

Methods

Coating of Carriers (Optional)

a. Bring a single CelCradle™ bottle into a BSC Class II hood
b. Coat carriers with 0.01% poly-L-lysine (PLL) (Sigma P4707); 30 min; RTP
c. Suction
d. Briefly rinse the carriers with 250 ml PBS by swirling the bottle gently
e. Suction
f. Allow carriers to completely dry inside the hood overnight

Seed Thaw/Expansion

Day -X: Preparation of Inoculum

a. Use cells with low passage number
b. Frozen aliquot should be passaged at least twice before using
c. Passage cells when confluency reaches no more than 85% at 1:5-8 ratio

Note: Do not over trypsinized cells. It causes damage to cell surface proteins which may result to sub-optimal attachment onto carriers

CelCradle Inoculation

Day 0: Seeding of Cells

a. Prepare fresh 150 ml of media inoculated with $0.75x10^8$ cells.
b. Pour cell suspension into a CelCradle500A (CC) bottle
c. Compress bellow to remove as much air from vessel before sealing tightly with the white cap provided
d. Invert CC bottle and ensure all carriers are properly submerged in cell slurry.
e. Place CC bottle into CO_2 incubator set at 5 % CO_2; 37°C
f. For every 30 min, gently swirl bottle to allow cells that have not yet attached onto carriers to mix homogenously across solution for further seeding.
g. Repeat for the first 3-5 hours of the seeding process.
h. After 3 hours of incubation, bring the CC bottle into the BSC. Gently mix the slurry and the carriers, and sample media for cell counting. Count suspended cells left in the culture media and determine percentage (%) of attachment.
i. If attachment rate of 80% is achieved, fill up CC bottle with fresh complete media to have a total volume of 500 ml and cap with the provided blue filter cap.

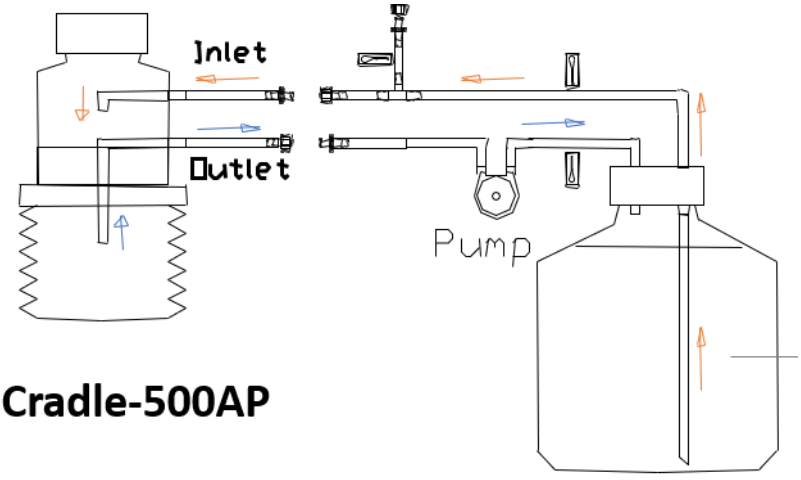
j. Place bottle onto platform and allow cells to adapt/grow overnight using the following parameters:
   i. Uprate: 1 mm/sec
   ii. Uphold: 1 min
   iii. Downrate: 1 mm/sec
   iv. Downhold: 1 min

OR

incubate an additional hour and check attachment efficiency. Typically, cell attachment is completed within 3-5 hours; attachment efficiency will not improve after 5 hours.

Perfusion Option of Culture (500AP)

a. If perfusion culture is opted, prepare:
   a. 2 L glass bottle containing 2.2 L of media
   b. Connect media bottle to CelCradle (500P or 500AP) and pump (See picture)
   c. Set program suited to your liking:
      i. Perfusion volume (standard as: 1999ml)
      ii. Day and date to perform perfusion
      iii. Frequency of perfusion: (standard as 24 cycles/day)



CelCradle-500AP

2L Perfusion bottle with 2.2L media

Monitoring Cell Growth

Daily monitor the residual glucose concentration and the pH of the culture medium to predict the time to change culture media or to supplement with extra glucose or sodium bicarbonate.

a. 2 ml media: pH and glucose measurement
b. (Optional)
   3 carriers: metabolic activity using PrestoBlue Assay following standard protocol
c. 3 carriers: live/dead cell staining using FDA, PI, Hoechst stains following standard protocol
d. 10 carriers: harvest using trypsin to check for live cell count (see harvest protocol)
e. Change with fresh media when:
    a. Glucose level falls below 1g/L
f. Maintain pH > 6.80
    a. If pH drops to 7.10, lower CO2%. If CO2% is already at zero, pulse in small amounts of sodium bicarbonate solution (intervals of 0.1g/L), observe in another 3hours. Add more sodium bicarbonate if needed.

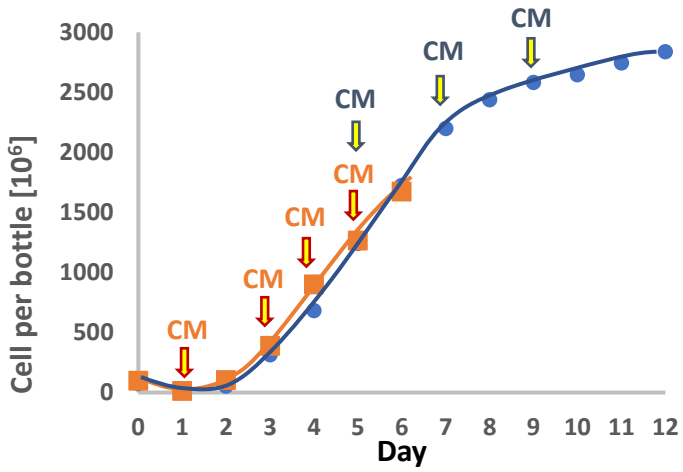
Cell Harvest

a. Drain off culture media into a container using plastic sieve
b. Rinse with 250 ml of PBS gently by inverting bottle with white cap, allowing carriers to be immersed in PBS solution with gently rolling motion for 20 sec
c. Drain
d. Repeat PBS wash
e. Drain
f. Pour 120-150 ml of pre-warmed 0.05% trypsin solution into the bottle and cover it with a white cap (without air vent)
g. Invert the bottle upside down to allow carriers to be soaked with the pre-warmed trypsin solution
h. Place bottle into 37˚C incubator for 15-20 min
i. Neutralize by adding 15 ml of 100% FBS
j. With the white cap on, hold the bottle in its inverted position then vigorously shake and struck the bottle against your palm for 1 min (see video attachment)
k. Harvest cell slurry using sieve
l. Repeat harvest using 100 ml of complete media for 4 more times
m. Pool all harvests together
n. Spin at 400x g in a pre-cooled centrifuge machine for 10 min
o. Suction supernatant

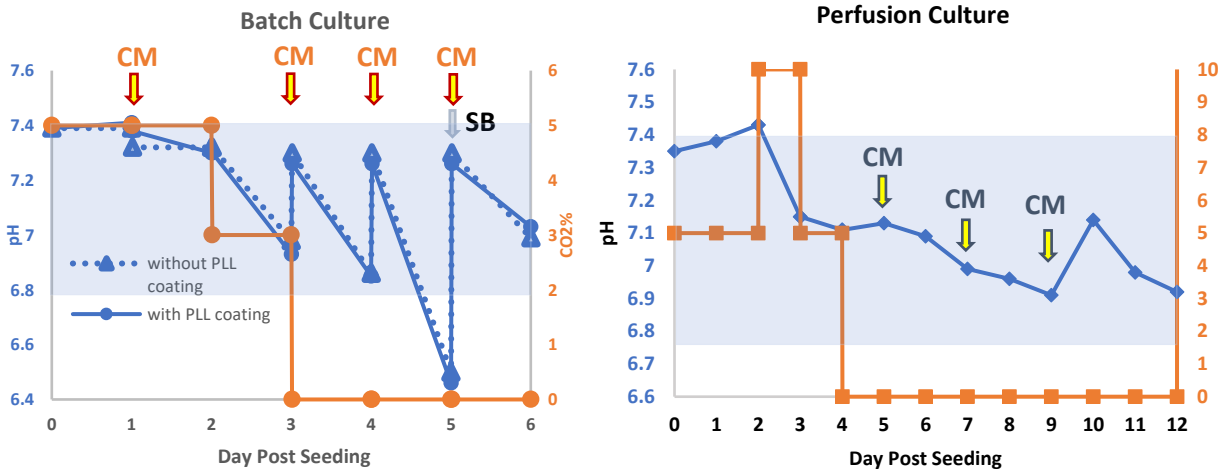
p. Resuspend pellet with complete media
q. Live count cells
r. Proceed to the next intended step for seeding cells into TideXcell-002 vessel or perform high cell density banking.

Results

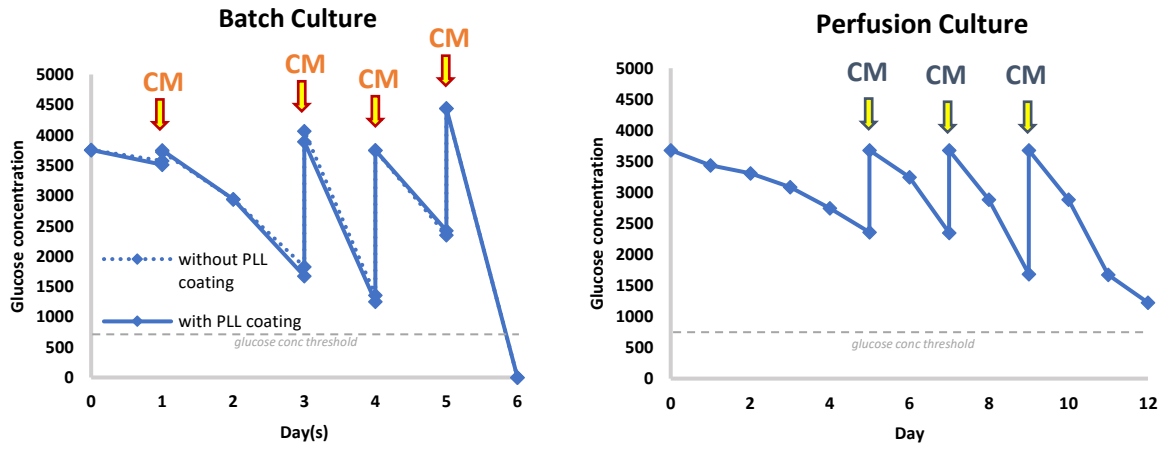
Cell Culture Profile (orange: batch; blue: perfusion)



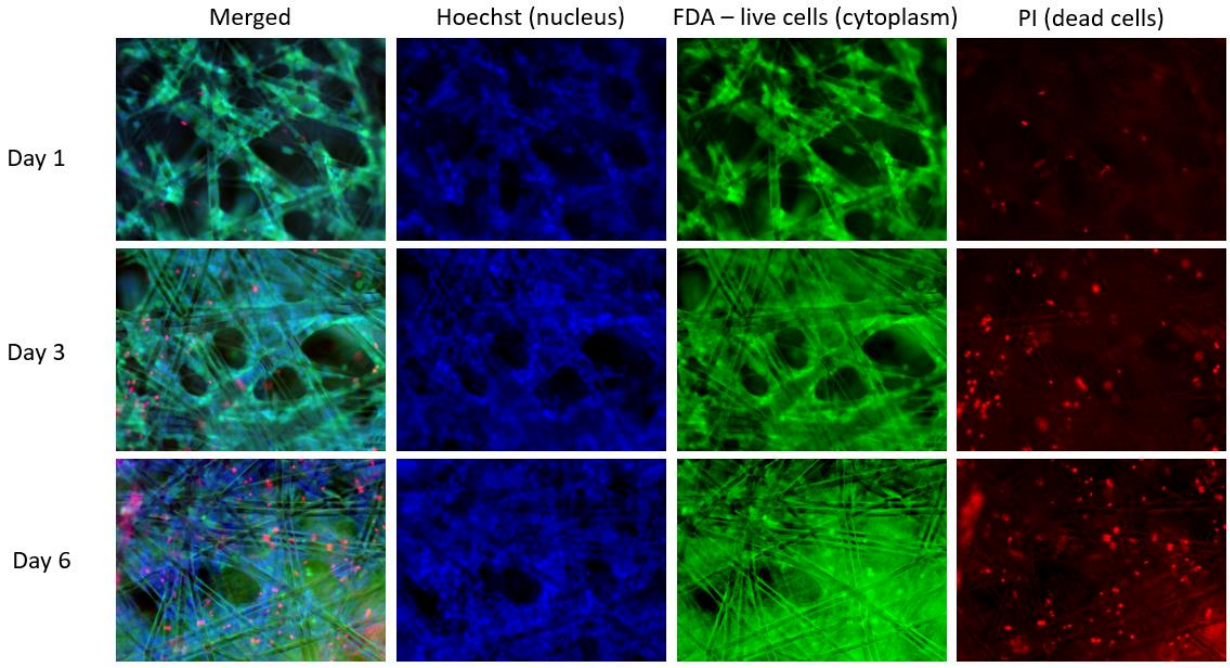
pH and CO2% Profile



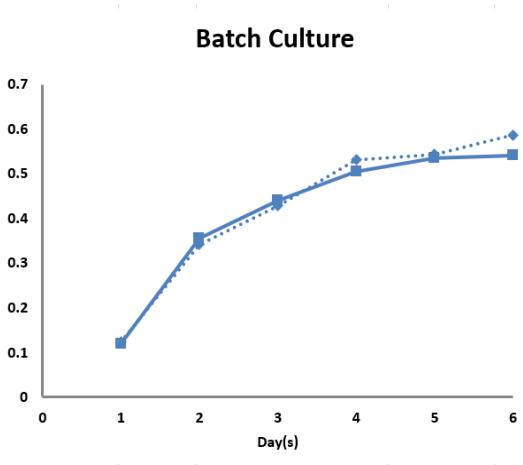
Glucose Profile

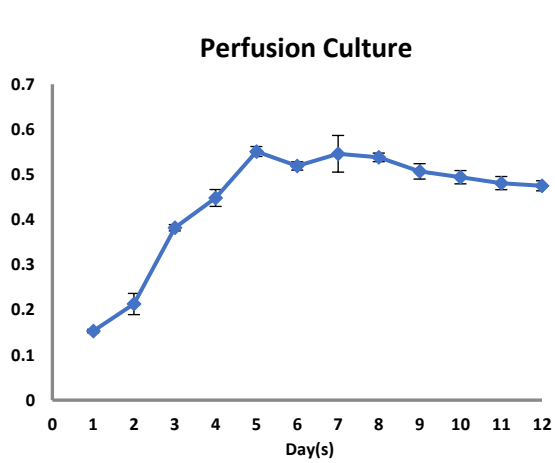


Cell Stains



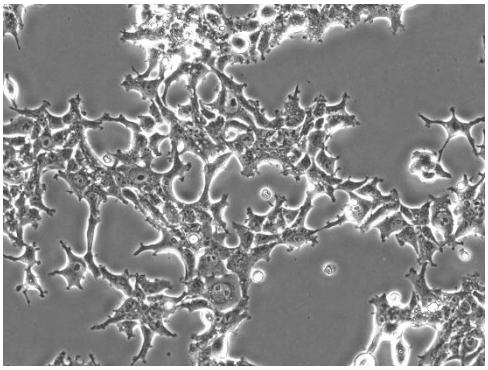
Cell Metabolic Activity



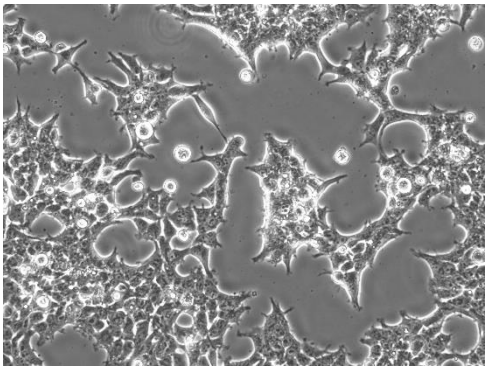


Cells Harvested from Macrocarriers Remained Morphologically Healthy

Cells used to prepare inoculate on Day 0



Cells harvested from CC (Day 6) reseeded onto 2D flasks



Cell harvest of >90% was obtained with cells maintaining at >95% viable. Cells harvested from CelCradle were plated onto 2D vessel overnight. Healthy cell morphology was maintained.