Conditioned Medium Epithelial Cell Expansion Protocol

Catalog # 256-100

Description

Propagenix Conditioned Medium is used for in vitro expansion of primary non-keratinocyte epithelial cells derived from either normal or diseased tissues. It is generated from irradiated 3T3-J2 mouse fibroblast cells (Propagenix Catalog No. PF-1100) and CRM medium (Propagenix Catalog No. 276-101). The 3T3-J2 material used by Propagenix is derived using a clone obtained under an exclusive license granted to Propagenix for intellectual property created at Georgetown University in the laboratory of Professor Richard Schlegel. The Conditioned Medium contains nutrients, fetal bovine serum and growth factors required to support cell growth in the absence of growth-arrested feeder cells when supplemented with Cholera toxin. Cholera toxin should be purchased separately to supplement the medium. Antibiotics may be added to the Conditioned Medium if desired.

Intended Use

For Research Use Only.

Storage

1. Store Conditioned Medium at -200C until required.
2. When ready to use, thaw Conditioned Medium overnight at 2° to 8°C protected from light.
3. Once thawed, medium is stable for up to two weeks at 2° to 8°C.
4. If you think it is unlikely you will use the entire contents of the bottle within two weeks, we recommend aliquoting the medium into smaller containers and re-freezing one additional time before loss of activity is observed, storing new aliquots at -20°C. Prepare aliquots based upon anticipated use within 2 weeks.

Usage Protocol

This protocol describes the use of Conditioned Medium to expand human epithelial cells without the direct presence of irradiated feeder cells. At the start of the culture, thaw epithelial cells, count, and plate the cells onto tissue culture vessels. Change the Conditioned Medium every 2 – 3 days until the cells approach 80% – 90% confluence, when they are passaged into new culture vessels. Continue the process until the desired population doublings (PDs) or the desired biomass has been achieved.

Population doubling: Population doubling (PD) refers to the total number of times the cells in the population have doubled during the culture period. Use the following equation to determine the number of population doublings.

$$PD = 3.32 \ x \ log\left(\frac{[total\ cell\ number\ at\ harvest]}{[total\ viable\ cell\ number\ seeded\ at\ the\ beginning\ of\ culture]}\right)$$

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Reagents required

1. Human epithelial cells for expansion.
2. Conditioned Medium, (Propagenix cat # 256-100). The medium should be stored at -20°C for long-term storage.
3. 1 mg/mL Cholera toxin (Sigma cat # C8052 or Enzo cat # BML-G117-0001).
4. 0.25% Trypsin-EDTA, e.g., Thermo Fisher Scientific, Cat# 25200-056.
5. Ca^{2+}, Mg^{2+} free PBS, e.g., Thermo Fisher Scientific, Cat# 10010-023.
6. DMSO for cell cryopreservation, e.g., Sigma, Cat# D2650.
7. CoolCell® Cryopreservation Alcohol-Free Cell Freezing Containers, BioCision®.

Procedure

I. Supplement Conditioned Medium

Prior to use, the CM must be completed by the addition of Cholera toxin: Sigma Catalog No. C8052 and Enzo Life Sciences Catalog No. BML-G117-0001 are recommended commercial sources for Cholera toxin.

1. Aseptically rehydrate Cholera toxin to a final stock concentration of 1 mg/mL in sterile distilled water or CRM.
    a. For example, 1mL of sterile distilled water may be used.
2. Aseptically add enough of the 1 mg/mL Cholera toxin stock solution to 100 mL of Conditioned Medium to achieve a final concentration of 8.6 ng/mL.
    a. For example, to make Complete Conditioned Medium containing a final concentration of 8.6 ng/mL Cholera toxin, add 0.9 µL Cholera toxin stock solution prepared in step 1 to 100 ml of Conditioned Medium.
3. If desired, prepare aliquots and store stock solutions of Cholera toxin at 2° to 8°C for up to six months.

II. Preparation of Conditioned Medium for use

1. Transfer desired amount of Conditioned Medium (CM) to a vented T-flask and place at 37°C in a 5% CO2 humidified incubator for 1 – 3 hours to allow the CM pH to equilibrate. Typically, 0.2mL CM per cm2 is adequate, e.g., use 5 mL of CM for a 25 cm2 flask.
2. Change the Conditioned Medium every 2 – 3 days until the cells approach 80% - 90% confluence. We recommend that you do not let the cells become fully confluent, it is likely to result in reduced expansion potential of the cultured cells.
3. Return remaining CM to the recommended storage conditions for up to two weeks at 2-8°C.

III. Thawing frozen epithelial cells

1. Thaw epithelial cells and initiate cell culture as soon as possible upon receiving the frozen cells. If not to be used right way, the cells should be stored in liquid nitrogen vapor phase.
2. Immediately prior to use, thaw the vial of epithelial cells by gentle agitation in a 37°C water bath. Thawing should be complete in approximately 2 – 3 minutes.
3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate it by spraying with 70% ethanol.
4. Transfer the vial contents to a 15 ml conical centrifuge tube containing 5 ml of warm CM. Centrifuge the cell suspension at approximately 200 x g for 5 minutes.
5. Aspirate most of the supernatant from the conical centrifuge tube containing the cell pellet. Flick the tube briefly to loosen the pellet, and then resuspend the cells in 0.5 – 1 ml warm CM.
6. Determine the viable cell number using an automated cell counter such as Countess II or a hemocytometer using standard Trypan Blue exclusion assay.
7. Seed epithelial cells between 4,000 – 10,000 viable cells/cm^2. Mix the desired numbers of epithelial cells with the CM to ensure a uniform suspension, and dispense the calculated volume into the culture flask. Use 1 ml CM for every 5 cm^2 surface area, e.g., use 5 ml CM for a 25 cm^2 flask.

   NOTE: Typically, in our hands most epithelial cells grow at a slower rate in Conditioned Medium than they do compared with their growth rate using Conditional Reprogramming Medium (Propagenix Catalog No. 276-101) in co-culture with growth-arrested 3T3-J2 feeder cells (Propagenix Catalog No. PF-1100). This is normal and to be expected.

8. Incubate the cultures at 37°C in a 5% CO_2 humidified incubator.
9. Change the growth medium every 2 – 3 days. As the cells become more confluent, increase the volume of medium as follows: under 50% confluence feed 1 ml per 5 cm^2, over 50% confluence feed 1.5 ml per 5 cm^2.

IV. Sub-culture epithelial cells culture

The epithelial cells culture should be split when the cells approach 80% – 90% confluence. **DO NOT** allow the cells to reach complete confluence, as this will induce the cells to differentiate and significantly decrease their ability to continuously replicate.

Volumes used in this protocol are for a 25 cm^2 flask; proportionally reduce or increase the amount of media for culture vessels of other sizes.

1. Remove spent medium.
2. Add 3 – 5 ml PBS to the flask. Briefly rinse the cells and discard the rinse solution.
3. Add 1 ml warm 0.25% Trypsin-EDTA to the flask. Incubate at 37°C for 5 min, or longer time if necessary, to detach the cells. Tap the flask several times against your palm to loosen the cells.

4. Neutralize the trypsin by adding 2 ml warm CM and pipette up and down several times to break up any cell clumps into suspension.
5. Transfer the cell suspension into a sterile 15 ml centrifuge tube. Rinse the flask with an additional 1 – 2 ml CM and pool into the centrifuge tube with cells.
6. Centrifuge cells at 200 x g for 5 min at room temperature.
7. Aspirate most of the supernatant from the centrifuge tube containing the cell pellet. Flick the tube briefly to loosen the pellet.
8. Dilute the cells in 1 ml of warm CM. Determine cell count and viability using an automated cell counter such as Countess II, or a hemocytometer using Trypan Blue.
9. Use the following equation to determine the number of population doubling (PD).

$$PD = 3.32 \quad x \quad \log\left(\frac{[total\ cell\ number\ at\ harvest]}{[total\ viable\ cell\ number\ seeded\ at\ the\ beginning]}\right)$$

10. Seed between 4,000 – 10,000 viable cells/cm^2 into a new subculture vessel(s). Mix desired number of epithelial cells in the CM to ensure a uniform suspension, and dispense the calculated volume into the subculture flasks. Use 5 ml CM for a 25 cm^2 flask.
11. Incubate the cultures at 37°C in a 5% CO_2 humidified incubator.
12. Change the growth medium every 2 – 3 days. As the cells become more confluent, increase the volume of media as follows: under 50% confluence feed 1 ml per 5 cm^2; over 50% confluence feed 1.5 ml per 5 cm^2.

V. Cryopreservation of epithelial cells

1. Cryopreservation Medium: CM, 90% (v/v); DMSO, 10% (v/v).
2. Follow the subculture protocol to harvest epithelial cells when they approach 80% – 90% confluence.
3. Determine cell count and viability using an automated cell counter such as Countess II or a hemocytometer using Trypan Blue.
4. The cells can be frozen at a density of 0.5 – 5 x 10^6 cells/ml. Resuspend cells in an appropriate amount of cryopreservation medium, and aliquot into cryovials.
5. Put the cryovials into a CoolCell™ and leave it in a -80°C freezer for at least 2 hours or overnight.
6. Transfer the cells to the vapor phase of liquid nitrogen for long-term storage.

Quality Assurance

1. CM is tested prior to shipment to meet quality control specifications. Each lot is tested for sterility, pH, visually inspected for appearance and functionally tested to support the growth of epithelial cells.
2. Additional information is available upon request.

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Limited Use Label License (LULL)

Conditional Reprogramming (CR) Technology, covered under US Patent No. 9,279,106 (issued March 8, 2016), and subsequent patent applications pending in the US and other Jurisdictions.

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