General Instructions for Culturing

Canine Trabecular Meshwork Cells (CnTMC)

Open all the packages immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.

I. STORAGE

A. CRYOPRESERVED VIALS (Cn634-05)
   Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. PROLIFERATING FLASKS (Cn635-)
   1. Examine under a microscope to check if all the cells are attached to the bottom of the flask. If not, notify CAI or your distributor immediately.
   2. Decontaminate the exterior of the cell culture flask with 70% alcohol.
   3. Place the sealed flask in a 37°C, 5% CO₂ humidified incubator for 2 hours as shipped.
   4. In a sterile biological safety cabinet, open the cap of the flask very slowly and carefully.
   5. Remove the Transport Medium by aspiration. Add fresh Growth Medium: 5 ml for a T-25 flask and 15 ml for a T-75 flask.
   6. Place the flask in a 37°C, 5% CO₂ humidified incubator with loosened cap to allow gas exchange.
   7. Change medium every other day.

C. GROWTH MEDIUM (Cn631-500)
   Store the Growth Medium at 4°C in the dark immediately upon arrival.

D. SUBCULTURE REAGENT KIT (090K)
   Store at -20°C immediately upon arrival.
   Store at 4°C after thawing.

II. PREPARATION FOR CULTURING

1. Make sure the Class II biological safety cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the biological safety cabinet with 70% alcohol to ensure it is sterile.
3. Turn the biological safety cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with cell cultures.
   c. Handle all cell culture work in a sterile biological safety cabinet.

III. CULTURING CnTMC

A. PREPARING CELL CULTURE FLASKS FOR CULTURING CnTMC
   1. Take the Canine Trabecular Meshwork Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile biological safety cabinet.
   2. Pipette 15 ml of Canine Trabecular Meshwork Cell Growth Medium* into a T-75 flask.

* Keep the medium to surface area ratio at 1 ml per 5 cm². For example, 5 ml for a T-25 flask or a 60 mm tissue culture dish. 15 ml for a T-75 flask or a 100 mm tissue culture dish.

Cell Applications Inc (hereinafter CAI) warrants that its products are manufactured with the utmost care and stringent quality control procedures. However, if you should ever have a problem with the products, we will either replace the products, or in the case we cannot deliver the products, provide you with a refund. Such warranty is applicable only when CAI’s cells are used in conjunction with CAI’s medium and subculture reagents, and vice versa.
B. PREPARING CULTURE FLASK
1. Take the Canine Trabecular Meshwork Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile biological safety cabinet.
2. Pipette 35 ml of Canine Trabecular Meshwork Cell Growth Medium to a T-175 flask (to be used in Section IV C Step 15).

C. SUBCULTURING CnTMC
Trypsinize Cells at Room Temperature. Do Not Warm Any Reagents to 37°C.
1. Remove the medium from culture flasks by aspiration.
2. Wash the monolayer of cells with HBSS and remove the solution by aspiration.
3. Pipette 5 ml of Trypsin/EDTA Solution into the T-75 flask. Rock the flask gently to ensure the solution covers all the cells.
4. Remove 4 ml of the solution immediately.
5. Re-cap the flask tightly and monitor the trypsinization progress at room temperature under an inverted microscope. It usually takes about 2 to 5 minutes for the cells to become rounded.
6. Release the rounded cells from the culture surface by hitting the side of the flask against your palm until most of the cells are detached.
7. Pipette 5 ml of Trypsin Neutralizing Solution to the flask to inhibit further trypsinic activity.
8. Transfer the cell suspension from the flask to a 50 ml sterile conical tube.
9. Rinse the flask with an additional 5 ml of Trypsin Neutralizing Solution and transfer the solution into the same conical tube.
10. Examine the T-75 flask under a microscope. If there are >20% cells left in the flask, repeat Steps 2-9.
11. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
12. Aspirate the supernatant from the tube without disturbing the cell pellet.
13. Flick the tip of the conical tube with your finger to loosen the cell pellet.
14. Resuspend the cells in 2 ml of Canine Trabecular Meshwork Cell Growth Medium by gently pipetting the cells to break up the clumps.
15. Count the cells with a hemocytometer or cell counter. Inoculate at 9,000 cells per cm² for rapid growth, or at 6,000 cells per cm² for regular subculturing.