

General Instructions for Culturing

Human Follicle Dermal Papilla Cells (HFDPC)

Be sure to wear face protection mask and gloves when retrieving cryovials from the liquid nitrogen storage tank. The dramatic temperature change from the tank to the room could cause any trapped liquid nitrogen in the cryovials to burst and cause injury.

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 (602-05a, 602t-05a)

Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. PROLIFERATING FLASKS (603-25a, -75a)

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 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 (611-500)

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.

E. COLLAGEN COATED T-75 FLASK (125-75)

Store at 4°C immediately upon arrival.

F. COLLAGEN SOLUTION (125-50)

Store at 4°C temperature.

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 human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
 - c. Handle all cell culture work in a sterile hood.

III. CULTURING HFDPC

A. PREPARING CELL CULTURE FLASKS FOR CULTURING HFDPC

1. Take the Collagen Coated Flasks and Papilla Cell Growth Medium from the refrigerator and allow them to warm to room temperature.
2. Wipe the bottle and flask with 70% alcohol in a sterile hood.
3. Pipette 15 ml of Papilla Cell Growth Medium* to the Collagen Coated T-75 flask.

* Use Corning and Grenier flasks for best results.

* Keep the medium to surface area ratio at 1-1.5ml per 5 cm².
For example,
7.5 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.

B. THAWING AND PLATING HFDPC

1. Prepare a 15ml sterile conical tube for washing HFDPC by adding 12ml of Papilla Cell Growth Medium
2. Remove the cryopreserved vial of HFDPC from the liquid nitrogen storage tank using proper protection for your eyes and hands.
3. Thaw the cells quickly by placing the lower half of the

vial in a 37°C water bath and watch the vial closely during the thawing process.

4. Take the vial out of the water bath when only small amount of ice left in the vial. Do not let cells thaw completely.
5. Take the vial out of the water bath and wipe dry.
6. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
7. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
8. Disperse the cells in the vial by gently pipetting the cells 5 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
9. Pipette the cell suspension (1ml) from the vial into the prepared 15ml conical tube gently.
10. Centrifuge the cells at 200 x g for 5 minutes at room temperature to pellet the cells.
11. Aspirate the supernatant from the tube without disturbing the cell pellet.
12. Resuspend the cells in 2ml of Papilla Cell Growth Medium by gently pipetting the cells to break up the clumps.
13. Transfer the 2ml cell suspension to the Collagen Coated T-75 flask containing 15ml of Papilla Cell Growth Medium.
14. Replace the cap tightly and swirl the flask gently to distribute the cells evenly in the flask.
15. Place the T-75 flask in a 37°C, 5% CO₂ humidified incubator. Loosen the cap to allow gas exchange.
16. Change Papilla Cell Growth Medium every other day until the cells reach 60% confluent.
17. Double the Papilla Cell Growth Medium volume when the culture is >60% confluent or for weekend feedings.
18. Subculture the cells when the HFDPC reach 80% confluent.

IV. SUBCULTURING HFDPC

A. PREPARING SUBCULTURE REAGENTS

1. Remove the Subculture Reagent Kit from the -20°C freezer and thaw overnight in a refrigerator.
2. Make sure all the subculture reagents are thawed. Swirl each bottle gently several times to form homogeneous solutions.
3. Store all the subculture reagents at 4°C for future use. The activity of Trypsin/EDTA Solution will be stable for 2 weeks when stored at 4°C.
4. Aliquot Trypsin/EDTA solution and store the unused portion at -20°C if only portion of the Trypsin/EDTA is needed.

B. PREPARING CULTURE FLASK

1. Swirl the Collagen Solution bottle a few times to form a homogenous solution.
2. Take the Papilla Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
3. Add 10ml of Collagen Solution to a T-225 flask and rock the flask gently to distribute the solution evenly over the

whole culture surface.

4. Coat the culture ware for 1-2 hours at room temperature.
5. Remove the Collagen Solution by aspiration in a sterile hood.
6. Wash the coated flask three times with PBS and aspirate PBS completely from the flask in the last wash. The coated flask can be used immediately or stored in a sealed bag at 4°C for up to 2 weeks.
7. Prepare the coated flask for subculturing by pipetting 45ml of Papilla Cell Growth Medium into this coated T-225 flask and wait to be seeded.

C. SUBCULTURING HFDPC

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 4.5 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 1 to 3 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 tryptic 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 Papilla 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 10,000 cells per cm² for rapid growth, or at 6,000 cells per cm² for regular subculturing.