Protocol cell culture and 5G FR2 exposure

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Cell culture of primary human skin cells

This protocol describes the maintenance and storage of primary human keratinocytes and melanocytes. The primary human skin models are:

- Primary human epidermis keratinocytes from juvenile male foreskin and adult female eyelid (NHEK-f.c. and NHEK-c., respectively)
- Primary human epidermis melanocytes from adult female eyelid (NHEM).

The cells were obtained from healthy donors. Frozen cryovials were purchased from PromoCell, Germany. A working batch were prepared and stored in cryotank at passage 3.

The doubling time was measured at 24.6 h for NHEK-f.c., 23.4 h for NHEK-c. and 80 h for NHEM.

Preparation of working batch

- Pre-warm 15 ml growth medium in a T75 cell culture flask to 37 °C.
- Thaw original cryovial from PromoCell in a water bath at 37 °C until the suspension detached from vial wall.
- Quickly transfer the cell suspension into the pre-warmed flask.
- Incubate at 37 °C and 5% CO_2.
- After 24 h, replace the medium using 15 ml pre-warmed growth medium.
- After 2 days, replace the medium using 15 ml pre-warmed growth medium.
- On day 7, detach the cells according to trypsinization section.
- Instead of resuspending the cell pellets using growth medium after centrifugation, resuspend using CryoSFM Plus medium.
- Aliquot 1 ml cell suspension in cryovials.
- Freeze the cryovials using Planer Kryo 10 Series II.
- Store the frozen aliquots in a cryotank until further use.

Trypsinization

- Dissolve the trypsin inhibitor in DPBS without Ca and Mg to reach a concentration of 0.125 mg /50 µl PBS.
- Sterile-filter the dissolved trypsin inhibitor using 20 µm filter.
- Pre-warm 15 ml growth medium in T75 cell flask or 5 ml in T25 flask (as needed).
- Pre-warm 8 ml growth medium in a centrifuge tube.
- Wash the cells using 4 ml pre-warmed DPBS without Ca and Mg twice.
- Add 2 ml trypsin to the flask and incubate at 37 °C for 2 min.
- Quickly add 100 µl trypsin inhibitor after incubation and 8 ml growth medium.
- Transfer the cell suspension into a 10 ml centrifuge tube.
- Take 100 µl sample for cell count using CASY automatic cell counting system.
- Centrifuge the cell suspension at 800 rpm for 5 min.
- Remove the supernatant and add growth medium to reach the desired cell density.
- Transfer needed cell suspension into the prepared cell flasks.

Cell culture

To be performed 5 days (NHEK-f.c. & NHEK-c.) or 10-14 days (NHEM) prior to exposure to reach the desired cell number for RF exposure.

- Pre-warm 15 ml growth medium in a T75 cell culture flask to 37 °C.
- Thaw the cells cryovial from working batch in a water bath at 37 °C until the cell suspension detached from vial wall.
- Quickly transfer the cell suspension into the pre-warmed flask.
- After 24 h, replace the medium using 15 ml pre-warmed growth medium.
- On fifth day, split the cells according to trypsinization section in T25 cell culture flasks.
    - Cell density: 5E+05/flask for 4 h exposure or 3.75E+05/flask for 24 h exposure.

A minimum of 3 T25 cell flasks per cell type is needed for an experiment.

5G FR2 exposure

The cells are exposed to fifth generation (5G) of mobile telephony in the frequency range 2 (FR2) at 27.5 GHz with 100 MHz bandwidth using the exposure system developed by IT'IS Foundation, Zürich, Switzerland. The system comprises a computer-controlled system rack with RF source, power supplies, and data acquisition, as well as three shielded chambers to operate in three different power density (see picture below):

- High: 10 W/m^2
- Low: 3.33 W/m^2
- Sham exposure

The built-in sensors monitor and record the conditions and control exposure, such as temperature and fan current for airflow needed for the cell culture. Each shielded chambers can contain 4 T25 flasks inserted directly into an internal support structure.

The cells are exposed in a blinded experiment, where the power density of each chamber is selected randomly by the computer and hidden during the exposure. Decoding of the blinded experiment is done by IT'IS Foundation after finishing assay analysis.

Cell exposition procedure

- After 24 h, replace the medium with pre-warmed 8 ml growth medium.
- Turn on the sxc mmWave exposure system.
- Put the cell flasks in the exposure chambers. If there are <4 flasks with cells to be exposed in each chamber, put additional T25 flasks filled with 8 ml medium to the empty slots.
- Start the sXRFc controller:
    - Fill the exposure duration (4 or 24 h) in the "Experiment Settings" with 30 min delay to allow the incubator to warm-up after inserting the cell culture flasks.
    - Set the power density for 3.33 W/m^2 (low) and 10 W/m^2 (high).
    - Click on "Start" field.
- At the end of exposure, remove the cell culture flasks and switch off the exposure system.
- Proceed with the cell harvest and assay.

Chemicals & materials

Chemicals

Chemicals	Provider (article number)
Keratinocytes Growth Medium 3	PromoCell (C-20021)
Melanocyte Growth Medium M3	PromoCell (C-24310)
CryoSFM Plus	PromoCell (C-29922)
Trypsin 0.05 %/EDTA 0.02 % in DPBS, w/o: Ca and Mg	PAN-Biotech (P10-023100)
DPBS, w/o: Ca and Mg	PAN-Biotech (P04-36500)
Trypsin inhibitor	Sigma Aldrich (T9003)

Materials

Material	Provider
T75 flasks	TPP
T25 flasks	TPP
Serological pipette (5, 10 & 25 ml)	Sarstedt
PIPETBOY acu 2 pipette controller	Integra
Centrifuge tube (15 & 50 ml)	Corning
Phase-contrast microscope	Olympus
Centrifuge Allegra V-15R	Beckman Coulter
Cell culture incubator with CO_2	Heraeus
Water bath	Thermo Fischer
Cryovials	Thermo Fischer
Planer Kryo 10 Series II	Messer Griesheim
CASY automatic cell counting system	Omni Life Science
Exposure incubator with CO_2	Binder
Sxc mmWave exposure system	IT'IS Foundation