Life Technologies™
Your Molecular & Cell Technology Partner

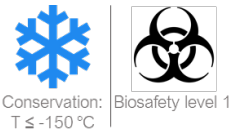
Human Sebocytes

Product sheet, catalog n° CTICC.1.4.1

cti BIOTECH

General Information

- Organism: Human (*Homo sapiens*)
- Tissue: Skin
- Cell Type: Skin Human Sebocytes from single donor
- Location: Abdomen or other part (see Certificate of Analysis)
- Gender: Male or Female (see Certificate of Analysis)
- Age: (see Certificate of Analysis)
- Phototype: (see Certificate of Analysis)



Cell Characteristics

- Cell properties: Adherent cells
- Morphology: Variable
- Isolation: From sebaceous glands
- Cell passage: (see Certificate of Analysis)
- Minimum number of population doublings: 15, it is recommended not to exceed Passage n°6
- Cell viability: Minimum 80% viability when thawed from cryopreservation
- Cell conditioning: Supplied as vials of 1M cells
- Cryopreservation medium: Frozen with 95% serum-free cryopreservation medium + 5% DMSO
- Storage condition: Liquid nitrogen
- Batch specific information: (see Certificate of Analysis)

Safety and Quality Control

- Biosafety level: 1
- Contamination: Use mandatory laboratory protections and handle with care tissues and cells derived from human samples to avoid any contamination of the operator
- Viral testing: negative for HIV, HBV, HCV
- Sterility testing: Negative for mycoplasma, bacteria and yeasts

Handling upon delivery and storage

- Check that the containers are intact and free of damage
- If cells are not used immediately, place the vials at -150°C or below upon delivery

Growth medium

- Recommended medium reference: Seb4Gln: Sebocyte growth and differentiation medium

Thawing and culturing procedure for frozen cells

*Note : **seeding of sebocytes (post thawing or when subculturing) is promoted by supplementation with human fibronectin***
Option 1: Fibronectin pre-coated flask may also be used for to support sebocytes adhesion
1 - Add 0.10-0.12 ml per cm² of Seb4Gln medium in a fibronectin coated tissue culture flask. (Recommended: 9mL in a T75 flask or 18mL in a T175 flask)
2 - Add 13 ml of PBS solution to a 15 ml conical tube, and warm in a water bath to 37 ˚C
3 – Start thawing cryovial by swirling in a water bath at 37 ˚C. As soon as the content has thawed, start step 4
4 - Once almost completely thawed, add the cell suspension to the warmed PBS in sterile conditions
5 - Spin the tube at 300 g for 7 minutes at 20°C to pellet the cells
6 - Resuspend the cells in the appropriate volume of recommended medium
7 – Seed the cells in the culture vessel at a concentration of 2 500 to 3 000 cells per cm²
8 - Incubate at 37˚C, 5% CO2 atmosphere, 95% humidity
9 - After 24 hours of incubation, change the medium to remove any debris
10 - Continue to incubate and change the medium every 3-4 days

Option 2: Make up the appropriate volume of seeding medium (recommended ratio: 21µL of 0.1% fibronectin solution => recommended Merck Sigma Aldrich Cat N°#F0895) per mL of Seb4Gln medium.
1 - Add 0.10-0.12 ml per cm² of seeding medium in a fibronectin-coated tissue culture flask. (Recommended: 9mL in a T75 flask or 18mL in a T175 flask)
2 - Add 13 ml of PBS solution to a 15 ml conical tube, and warm in a water bath to 37 ˚C
3 - Thaw cryovial by swirling in a water bath at 37 ˚C. As soon as the content has thawed, start step 4
4 - Once thawed, add the cell suspension to the warmed PBS in sterile conditions
5 - Spin the tube at 300 g for 7 minutes at 20°C to pellet the cells
6 - Resuspend the cells in the appropriate volume of recommended medium
7 – Seed the cells in the culture vessel at a concentration of 2 500 to 3 000 cells per cm²
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Subculturing

1- Start sub-culturing when cells reached 90%-100% confluence
2 - Preheat TrypLE (non-toxic for cell - trypsin substitute) and recommended medium
3 - Remove the medium from the flask
4 - Wash the cells quickly with PBS without Ca2+ Mg2+
5 - Add 0.06mL per cm² of TrypLE for 5 min at 37 °C in the incubator
6 - Remove the cells from the flask by pipetting several times and wash the flask with recommended medium for remaining cells
7 - Centrifuge the cells in recommended medium at 250 g for 7 minutes to pellet the cells
8 - Remove the supernatant and resuspend the pellet in recommended Seb4Gln medium.

Option 1: Fibronectin pre-coated flask may also be used for to support sebocytes adhesion
9 - Add 0.10-0.12 ml per cm² of Seb4Gln medium in a fibronectin coated tissue culture flask. (Recommended: 9mL in a T75 flask or 18mL in a T175 flask). Seed the cells in the culture vessel at a concentration of 2 500 to 3 000 cells per cm².
10 - Incubate at 37˚C, 5% CO2 atmosphere, 95% humidity
11 - After 24 hours of incubation, change half of the medium to remove any debris
12 - Continue to incubate and change the medium every 3-4 days

Option 2: Make up the appropriate volume of seeding medium (recommended ratio: 21µL of 0.1% fibronectin solution (recommended Merck Sigma Aldrich Cat N°#F0895) per mL of Seb4Gln medium.
9 - Add 0.10-0.12 ml per cm² of seeding medium in a fibronectin-coated tissue culture flask.
(Recommended: 9mL in a T75 flask or 18mL in a T175 flask)
Seed the cells in the culture vessel at a concentration of 2 500 to 3 000 cells per cm².
10 - Incubate at 37˚C, 5% CO2 atmosphere, 95% humidity
11 - After 24 hours of incubation, change half of the medium to remove any debris
12 - Continue to incubate and change the medium every 3-4 days

Associated products

- **CTICC.1.1.1:** Adult Human Dermal Fibroblasts, Cryopreserved, 10^6 cells
- **CTICC.1.2.1:** Adult Human Keratinocytes, Cryopreserved, 10^6 cells
- **CTICC.1.3.1:** Adult Human Melanocytes, Cryopreserved, 10^6 cells
- **SKIN BIOPSIES:** Fresh, Flash Frozen, FFPE, OCT-embedded
- **Seb4Gln:** Sebocyte growth and differentiation medium

Provisions

- Cells and tissues are intended for **research use only** and shall not be used for human trials, animal trials, or diagnostics
- **Consent**: the original tissues have been obtained after informed consent of the patient under the provisions required by French Law
- **Primary Human cells** are not immortalised cell lines and may not be continually subcultured

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