Materials and reagents

- **Primary Keratinocytes from patient 2.10^6** cells / vial (stored in liquid nitrogen)
- **Keratinocyte growth medium**
  - 490 mL DermaCult™ Keratinocyte Expansion Medium (Stemcell technologies 100-0501) (storage at 4°C)
  - 10 mL DermaCult™ Keratinocyte Expansion Supplement (50X) (storage at -20°C)
  - 500 µL Hydrocortisone Stock Solution (200X) (Stemcell technologies 07925) (storage at -20°C)

(Once the complete medium has been reconstituted, it should be stored at 4°C and consumed within 30 days.)

- **Phosphate Buffer Saline (PBS)** without calcium and magnesium (Eurobio Scientific CS1PBS01-01)
- **Sterile 15 mL conical tubes**
- **Trypsin-EDTA (0.25%) (Gibco 25200056)**
- **DMEM, high glucose, GlutaMAX™ supplement, pyruvate (Gibco 31966047)**
  - 10% Foetal Bovine Serum (FBS)
  - 1% Penicillin-Streptomycin (PS)
- **Tissue-culture treated flasks, plates, or Petri dishes**
- **37°C Water Bath**
- **37°C Incubator with 5% CO_2 and 100% Humidity**

Thawing keratinocytes

1. **Prepare Culture Medium:** Pre-warm complet DermaCult™ Keratinocyte Expansion Medium to **37°C**.
2. **Thaw Cells**
   - Remove the vial from liquid nitrogen.
   - Quickly thaw in a **37°C water bath**, gently swirling the vial.
   - Do not submerge the cap; thaw within **< 2 minutes**.
3. **Transfer Cells**
   - Transfer the cells into a **15 mL tube**.
   - Rinse the vial with **1 mL of growth medium** and add dropwise to the cells.
   - Slowly add **8 mL of growth medium**, swirling gently.
4. **Centrifugation:** Spin at **200 × g for 5 min** at room temperature.
5. **Resuspend Cells:** Aspirate the supernatant and resuspend in **2 mL of fresh culture medium**.
6. **Plating Cells:** Seed all the resuspended cells in a T75 flask qsp 12mL of medium.
7. **Incubation:** Place in a **37°C, 5% CO_2 incubator**.
8. **Media Change:** Replace **the medium every 2–3 days**.
9. **Pass cells when they attend 70% of confluency.**

Primary keratinocyte passage

1. **Prepare Materials**
   - Warm **culture Medium** and **Trypsin-EDTA** to **37°C**.
2. **Aspirate medium**
3. **PBS Washing**
   - Rinse cells once with **PBS (Without Ca^{2+}, Mg^{2+})** (5 mL per **T25 flask**).
4. **Cell Detachment**
   - Add **1 mL of Trypsin-EDTA per T25 flask** (adjust for other dish sizes).
   - Incubate for **2–5 minutes at 37°C**, checking for detachment.
5. **Stop Reaction**
   - Add **5mL of DMEM Complet medium** to stop the trypsin reaction.
   - Gently pipette up and down to break clumps.
6. **Count cells using**
7. **Centrifugation**
   - Transfer 500 000 cells to a **15 mL tube**, spin at **200 × g for 5 min**.
8. **Resuspend Cells**
   - Use 5mL **pre-warmed keratinocyte growth medium**, mix gently, and count cells.
9. **Cell seeding**
   - Plate at **0.5 million cells per T25 Flask**.
   - Change **medium every 2–3 days**.

Growth Conditions

- **Temperature:** 37°C
- **CO_2 :** 5%
- **Humidity:** 100%