

	Reconstructed epidermis on polycarbonate membrane	
		Version 01 - 04/28/2025 Author : C. Cayron
		Page 1 / 2

Materials and reagents

- **Primary Keratinocytes from patient (2.45x10⁶ cells)**
- **Seeding medium**
 - 100 mL Epilife medium (Gibco MEPI500CA) (storage at 4°C)
 - 144 µL Ca²⁺ 1M (Sigma 21115-100ML) (storage at 4°C)
 - 100 µL Ascorbic Acide 50mg/mL (Sigma A4403)
 - 1 mL HKGS 100X (Gibco S-001-5) (storage at -20°C)
- **Differentiation medium**
 - 10 mL Epilife medium (Gibco MEPI500CA) (storage at 4°C)
 - 14.4 µL Ca²⁺ 1M (Sigma 21115-100ML) (storage at 4°C)
 - 10 µL Ascorbic Acide 50mg/mL (Sigma A4403)
 - 100 µL HKGS 100X (Gibco S-001-5) (storage at -20°C)
 - 10 µL KGF 10µg/mL (Sigma K1757) (storage at -20°C)
- **Phosphate Buffer Saline (PBS)** without calcium and magnesium (Eurobio Scientific CS1PBS01-01)
- **Sterile 15 mL conical tubes**
- **6 well plate**
- **Cell culture insert 0.4µm PCF, 12mm diameter** (Millipore PIHP01250)
- **37°C Water Bath**
- **37°C Incubator with 5% CO₂ and 100% Humidity**

Seeding of cells

1. Place one insert in the centre of each well in a 6 well pate
2. Add 2.5mL of seeding medium per well
3. Prepare a solution with 2.45x10⁶ keratinocytes in 3.5 mL of seeding medium
4. Distribute 0.5 mL of cell solution in each insert
5. Incubate cells for 48h at 37°C with 5% CO₂ in a humid atmosphere.

Epidermis differentiation at air/liquid interface

1. After 48h of incubation, aspirate medium in the insert and in the well.
2. Add 1.5mL of differentiation medium in each well (do not add any medium in the insert, to allow keratinocyte differentiation the insert should not contain any medium).
3. Incubate cells at 37°C with 5% CO₂ in a humid atmosphere.

Change medium in the well with differentiation medium every 2 to 3 days (1.5 mL/well).

Reconstructed epidermis could be use for experiment after 15 days post-seeding to 21 days post-seeding.

	Reconstructed epidermis on polycarbonate membrane	
		Version 01 - 04/28/2025 Author : C. Cayron
		Page 2 / 2

Growth Conditions

- **Temperature:** 37°C
- **CO₂ :** 5%
- **Humidity:** 100%