Human Keratinocyte 3D Culture

Catalog No.	Product Name	Product quantity	Short-term Storage	Long-term Storage	Thawing Instructions
ax3525	Keratinocytes (Pooled Donors)	500,000 cells/vial	Liquid nitrogen	Liquid nitrogen	See below
ax3528	Keratinocyte Cell Culture Medium	500 mL	4°C for 1 month	-20°C for 6 months	Thaw at 4°C or RT
ax3560	Keratinocyte 3D Culture Medium	500 mL	4°C for 1 month	-20°C for 6 months	Thaw at 4°C or RT
ax3570	Keratinocyte 3D Culture Starter Kit	1 kit	N/A	N/A	N/A
Included within ax3570	Polycarbonate Membrane Inserts (12 mm diameter) for 3D Culture	48 / pack	RT	RT	N/A
ax0044	Axol Unlock	25 mL	Aliquot and store at -80°C	-80°C	Thaw at 4°C

Lot-specific information such as donor details and passage number are stated in the Certificate of Analysis for each product.

Recommendations:

Always count the number of viable cells after thawing

- Recommended culture vessel coating: Not required
- Recommended cell culture medium: Axol Keratinocyte Cell Culture Medium followed by Axol Keratinocyte 3D Culture Medium
- Recommended seeding density: See below
- Recommended centrifugation speed: 200 x *g* for 5 min

Low passage and actively proliferating Keratinocytes must be used for successful 3D culture. The Keratinocytes should be cultured in Keratinocyte Cell Culture Medium for 2-3 passages before seeding for 3D culture

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Thawing & Plating:

- Thaw the **Keratinocytes (Pooled Donors)** quickly in a 37°C water bath until just prior to complete thawing.
- Wipe the outside of the vial with 70% ethanol.
- Gently resuspend the cells and take an aliquot to perform a cell count.
- Slowly dilute the cells into the required volume of pre-warmed **Keratinocyte Cell Culture Medium** (must be at least 10 mL so that the concentration of DMSO is less than 1%).
- Rinse the cryovial with 1 mL of **Keratinocyte Cell Culture Medium** to ensure all of the cells are transferred.
- Seed cells into the culture vessel at the recommended seeding density of **4,000 viable cells/cm^2**.
- Once the cells have attached (after 6-24 h), replace the culture medium with fresh, pre-warmed **Keratinocyte Cell Culture Medium**.
- Frequency of media changes: Every 2 days.

Passaging:

- Passage when the culture reaches: 70-80% confluent
- Recommended passaging reagent: Axol Unlock
- When the cells have detached from the culture vessel, dilute out the passaging reagent with **Keratinocyte Cell Culture Medium** and centrifuge the cells at 200 x *g* for 5 min.

It is important that the cells are centrifuged in order to remove the passaging reagent before plating the keratinocytes

- Remove the supernatant and resuspend in 1-2 mL of pre-warmed **Keratinocyte Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed **Keratinocyte Cell Culture Medium**.
- Seed cells into the culture vessel at the recommended seeding density of **4,000 viable cells/cm^2** for routine 2D culture or see below for 3D culture.

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3D Culture (Seeding into Inserts):

- When the keratinocytes are actively proliferating (approx. 4 population doublings per week), they are ready for 3D culture.
- Add 3 mL of **Keratinocyte Cell Culture Medium** to a 60 mm cell culture dish.
- Place 6 **Polycarbonate Membrane Inserts** into the culture dish to wet the insert membranes. Ensure no air bubbles are trapped under the inserts by slowly placing the inserts into the culture medium using forceps. Tilt the inserts slightly when adding to the culture medium.
- Prepare a cell suspension at **500,000 cells / mL** (0.5 x10^6 cells / mL) in **Keratinocyte Cell Culture Medium.**
- Add **400 µL** of cell suspension to each **Polycarbonate Membrane Insert** so that 200,000 cells (0.2 x10^6 cells) are seeded per insert.
- Add 11 mL of **Keratinocyte Cell Culture Medium** to the culture dish (not into the inserts). The levels of the culture medium in the dish and the inserts should equilibrate. Check that the keratinocytes are submerged in culture medium.
- Carefully place the culture dish into a humidified incubator at 37°C and 5% CO_2. Avoid moving the culture dish and inserts after seeding.
- Allow the cells to grow to confluency (typically 2 days after seeding).

It is essential that the cells are fully confluent before switching to 3D Culture Medium. If you are unsure, fix 1 insert and stain to assess confluency

- Optional: if you are unsure about the growth rate of the cells, transfer 1 insert to a 24-well plate and fix with 100% methanol. Stain the insert with Giemsa stain and assess cell confluency. If the keratinocytes have not reached confluency after 2 days, allow the cells in the remaining inserts to grow for another 1-2 days.

3D Culture (Switch to 3D Culture Medium):

- When the keratinocytes are fully confluent, they are ready to be switched into **Keratinocyte 3D Culture Medium**.
- Remove the **Keratinocyte Cell Culture Medium** from the culture dish and from inside the inserts.
- Add **400 µL** of **Keratinocyte 3D Culture Medium** to each insert.
- Add 11 mL of **Keratinocyte 3D Culture Medium** to the culture dish.
- Carefully place the culture dish into the incubator and leave overnight (for 15-16 h).

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3D Culture (Air-lift culture):

- Remove the **Keratinocyte 3D Culture Medium** from the culture dish and from inside the inserts.
- Transfer the inserts to new 60 mm dishes or a 24-well plate as required. Three inserts should be used per 60 mm dish. If one has been used to assess confluency, add a fresh insert without cells.
- For 60 mm dish culture, add **~3.2 mL** of **Keratinocyte 3D Culture Medium** to the culture dish so that the medium reaches the level of the membrane.
- For 24-well plate culture, add **~1 mL** of **Keratinocyte 3D Culture Medium** to each well so that the medium reaches the level of the membrane.
- Ensure no air bubbles are trapped under the inserts by slowly removing and replacing the inserts into the culture medium using forceps. Tilt the inserts slightly when adding to the culture medium.
- Carefully place the culture dish or plate into the incubator.
- The surface within the inserts should dry during air-lift and should remain dry throughout air-lift culture.
- Constant humidity is required for consistent 3D epidermis growth so check that the incubator is adequately humidified. Avoid opening the incubator door frequently. A separate humidified chamber could be used to maintain a constant humidity.
- For 60 mm dish culture, replace the **Keratinocyte 3D Culture Medium** every 2 days (e.g. Mon, Wed and Fri).
- For 24-well plate culture, replace the **Keratinocyte 3D Culture Medium** daily.
- Full thickness 3D epidermis takes 14-16 days to develop.
- For immunohistochemistry or immunofluorescence applications, the 3D epidermis can be fixed by transferring the insert into a 24-well-plate and incubating overnight with 4% Paraformaldehyde at 4°C. The membrane/epidermis can be cut out of the insert using a scalpel and can then be embedded in paraffin.

Usage Statement:

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

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