Human Keratinocytes
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Human Keratinocytes

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

**Recommendations**

*Important!*
Always count the number of viable cells after thawing.

- **Recommended culture vessel coating:** Not required
- **Recommended cell culture medium:** Keratinocyte Cell Culture Medium
- **Recommended seeding density:** 4,000 viable cells/cm²
- **Recommended centrifugation speed:** 200 x g for 5 minutes

**Thawing and Plating**

- Transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately to a 37°C water bath.
- Thaw the cells 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.
- **Immediately after thawing,** 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/cm².
- Incubate the cells at 37°C, 5% CO₂ in a humidified incubator.
- Once the cells have attached (after 6-24 hours), replace the culture medium with fresh, pre-warmed Keratinocyte Cell Culture Medium.
- Frequency of media changes: Every 2 days

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Product Name</th>
<th>Format</th>
<th>Short-term Storage</th>
<th>Long-term Storage</th>
<th>Thawing Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ax3525</td>
<td>Keratinocytes (Pooled Donors)</td>
<td>500,000 cell/vial</td>
<td>Liquid Nitrogen</td>
<td>Liquid Nitrogen</td>
<td>See below</td>
</tr>
<tr>
<td>ax3526</td>
<td>Keratinocytes (Single Donor, Juvenile)</td>
<td>500,000 cell/vial</td>
<td>Liquid Nitrogen</td>
<td>Liquid Nitrogen</td>
<td>See below</td>
</tr>
<tr>
<td>ax3527</td>
<td>Keratinocytes (Single Donor, Adult)</td>
<td>500,000 cell/vial</td>
<td>Liquid Nitrogen</td>
<td>Liquid Nitrogen</td>
<td>See below</td>
</tr>
<tr>
<td>ax3528</td>
<td>Keratinocyte Cell Culture Medium</td>
<td>500 mL</td>
<td>4°C for 1 month</td>
<td>-20°C for 6 months</td>
<td>Thaw at 4°C or RT</td>
</tr>
<tr>
<td>ax0044</td>
<td>Unlock</td>
<td>25 mL</td>
<td>Aliquot and store at -80°C</td>
<td>-80°C</td>
<td>Thaw at 4°C</td>
</tr>
</tbody>
</table>
Passaging

- Passage when the culture reaches: **70-80% confluent**
- Recommended passaging reagent: **Unlock**
- After adding passaging reagent, incubate the cells for **5 minutes** at **37°C**. Observe the cells at regular intervals for detachment from the culture vessel.
- Once the cells have detached from the culture vessel, dilute out the passaging reagent with Keratinocyte Cell Culture Medium.
- Centrifuge the cells at **200 x g** for **5 minutes**.

**Important!**

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/cm²**.
- Incubate the cells at **37°C, 5% CO₂** in a humidified incubator.

**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.

Got any questions? Need help with the protocol?
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Notes