Human Melanocytes

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Product Name</th>
<th>Product quantity</th>
<th>Short-term Storage</th>
<th>Long-term Storage</th>
<th>Thawing Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ax3529</td>
<td>Melanocytes (Single Donor, Juvenile)</td>
<td>500,000 cells/vial</td>
<td>Liquid nitrogen</td>
<td>Liquid nitrogen</td>
<td>See below</td>
</tr>
<tr>
<td>ax3530</td>
<td>Melanocytes (Single Donor, Adult)</td>
<td>500,000 cells/vial</td>
<td>Liquid nitrogen</td>
<td>Liquid nitrogen</td>
<td>See below</td>
</tr>
<tr>
<td>ax3531</td>
<td>Melanocyte Growth Medium</td>
<td>500 mL</td>
<td>4°C for 6 weeks</td>
<td>-20°C for 6 months</td>
<td>Thaw at 4°C or RT</td>
</tr>
<tr>
<td>ax3532</td>
<td>Melanocyte Differentiation Medium</td>
<td>250 mL</td>
<td>4°C for 6 weeks</td>
<td>-20°C for 6 months</td>
<td>Thaw at 4°C or RT</td>
</tr>
<tr>
<td>ax3542</td>
<td>Melanocyte Assay Medium</td>
<td>250 mL</td>
<td>4°C for 6 weeks</td>
<td>-20°C for 6 months</td>
<td>Thaw at 4°C or RT</td>
</tr>
<tr>
<td>ax0044</td>
<td>Axol 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>

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 Melanocyte Growth Medium for initial culture
  - Recommended seeding density: 4,000 viable cells/cm²
  - Recommended centrifugation speed: 200 x g for 5 min

**Thawing & Plating:**

- 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.
- Slowly dilute the cells into the required volume of pre-warmed Melanocyte Growth Medium (must be at least 10 mL so that the concentration of DMSO is less than 1%).
- Rinse the cryovial with 1 mL of pre-warmed Melanocyte Growth Medium to ensure all of the cells are transferred.
- Seed cells into the culture vessel at the recommended seeding density.
- Once the cells have attached (after 6-24 h), replace the culture medium with fresh, pre-warmed Melanocyte Growth Medium.
- Frequency of media changes: Every 2 days
Passaging:

- Passage when the culture reaches: 80% confluent
- Recommended passaging reagent: Axol Unlock
- When the cells have detached from the culture vessel, dilute out the passaging reagent with pre-warmed Melanocyte Growth 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 melanocytes.

- Remove the supernatant and resuspend in 1-2 mL of pre-warmed Melanocyte Growth Medium.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed Melanocyte Growth Medium.
- Seed cells into the culture vessel at the recommended seeding density.

Endpoint Assays:

- Our human Melanocyte Assay Medium has a lower concentration of growth factors and so reduces proliferation of the melanocytes. In the absence of growth factor stimuli, the melanocytes are more responsive to experimental conditions.
- Melanocytes should be cultured initially in Melanocyte Growth Medium until 95% confluent.
- Replace the culture medium with Melanocyte Assay Medium. The melanocytes can be maintained in Assay Medium for up to 1 week.

Differentiation:

- Our human Melanocyte Differentiation Medium has been optimized to promote melanocyte differentiation and increased melanin production.
- Melanocytes should be cultured initially in Melanocyte Growth Medium until 95% confluent.
- Replace the culture medium with Melanocyte Differentiation Medium. The melanocytes will undergo differentiation and will be fully differentiated in 5 days.

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.