Human iPSC-Derived Melanocytes

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0520	Human iPSC-Derived Melanocytes (Caucasian)	≥1 million cells/vial	N/A	Liquid nitrogen	N/A	N/A
ax0521	Human iPSC-Derived Melanocytes (African)	≥1 million cells/vial	N/A	Liquid nitrogen	N/A	N/A
ax0522	Human iPSC-Derived Melanocytes (Asian)	≥1 million cells/vial	N/A	Liquid nitrogen	N/A	N/A
ax0529	Melanocyte Proliferation Medium	1 x 100 mL Basal medium 2 x 200 µL Supplement	1x 250x	4°C -20°C	N/A Thaw at 4°C	Once combined 4°C for 1 week
ax3531	Melanocyte Growth Medium	500 mL	1x	4°C for 6 weeks	Thaw at 4°C	-20°C for 6 months

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Fibronectin	Sigma	F1141
TrypLE™ Express	Thermo Fisher	12605

Human iPSC-Derived Melanocytes behave like primary melanocytes in culture. **Melanocyte Proliferation Medium** has been optimized for high post-thaw survival rate (>85%) and amplification (at least 4 passages) on fibronectin substrate. However, **Human iPSC-Derived Melanocytes** will culture well in standard melanocyte culture medium such as, **Melanocyte Growth Medium**, although they will not be able to proliferate.

Preparation of Reagents

Melanocyte Proliferation Medium

- Upon receipt, store the **Melanocyte Basal Medium** at **4°C** and the **Supplement** at **-20°C**.
- Prepare complete **Melanocyte Proliferation Medium** by adding **200 µL Supplement** to **50 mL** of **Melanocyte Basal Medium**. Complete **Melanocyte Proliferation Medium** can be stored at **4°C** for **1 week**.

Melanocyte Proliferation Medium Contains Cholera Toxin, please handle according to necessary legislation and guidelines

Culture of Human iPSC-Derived Melanocytes

Coating

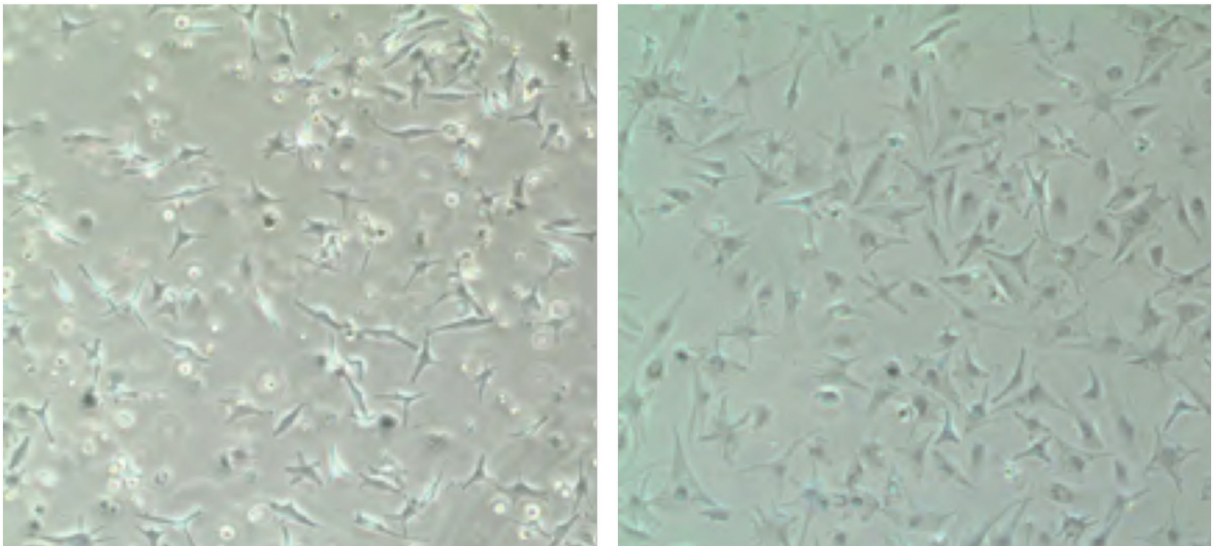
- We recommend coating the tissue culture plates with fibronectin diluted to 1/100 in 1x PBS. Incubate for at least **2 hours** in a **37°C** incubator.
- Before use, remove fibronectin coating solution.

Thawing and Plating

- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **Melanocyte Proliferation Medium** warm to **37°C** prior to use.
- To thaw the cells – transfer the vial of cells from storage by transporting the vial buried in dry ice. Remove the vial from dry ice and transfer it to a **37°C**, water bath.
- Quickly thaw the vial of cells in a **37°C** water bath. Do not completely submerge the vial (only up to 2/3rd of the vial). Remove the vial before the last bit of ice has melted, after **1-2 minutes**.
- **Do not shake the vial during thawing**.
- Take the vial of cells to a biological safety cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial in the hood.
- Using a P1000 pipette, gently add the cell suspension drop-wise into a 15 mL sterile conical tube containing **6 mL Melanocyte Proliferation Medium**.
- Perform a cell count to determine the number of viable cells and ensure optimal seeding density.
- Seed the cells on fibronectin-coated culture vessels at the recommended seeding density of **30,000 viable cells/cm^2**. Use **2 mL** of **Melanocyte Proliferation Medium** per 10 cm^2 of culture surface.
- To ensure an even plating of **Human iPSC-Derived Melanocytes** gently rock the culture vessel back and forth and side to side twice.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator until the cells have attached, approximately **4 hours**.
- Confirm that the **Human iPSC-Derived Melanocytes** before conducting a complete medium change with fresh pre-warmed, **37°C**, **Melanocyte Proliferation Medium**.

Maintenance

- Every other day, conduct a full medium change, use **2 mL** of **Melanocyte Proliferation Medium** per 10 cm^2 of culture surface.
- For weekends, use **3 mL** **Melanocyte Proliferation Medium** per 10 cm^2 of culture surface.
- Over the first 10-15 days, **Human iPSC-Derived Melanocytes** will gradually proliferate and pigmentation will become more prominent.
- Do not grow the **Human iPSC-Derived Melanocytes** to over confluency as it will decrease their survival after passaging.



Day 1 after thawing **Day 5 after thawing**

Figure 1: Proliferative Human iPSC-Derived Melanocytes will have a star-like morphology.
Human iPSC-Derived Melanocytes morphology will evolve after plating. Some floating cells may be observed one day after thawing.

Passaging

Human iPSC-Derived Melanocytes can be passaged up to 4 times after thawing. Passaging of **Human iPSC-Derived Melanocytes** can be performed every 7-15 days.

- Prepare culture vessels by coating with fibronectin at least 2 hours before thawing the **Human iPSC-Derived Melanocytes**.
- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **Melanocyte Proliferation Medium** and warm to **37°C** prior to use.
- Pre-warm TrypLE™ Express to **37°C**.
- Remove culture medium and wash the cells once with 1 x PBS.
- Add 1 mL TrypLE™ Express for each 10 cm^2 of culture surface.
- Incubate at **37°C**, **5% CO_2** for **5-10 minutes**. Regularly check the cells, when all the cells look rounded detach them by gently flushing with the culture medium present in the plate.

- Transfer the cells to a 15 mL sterile conical tube containing **Melanocyte Proliferation Medium** (anticipate at least a 1/3 dilution ratio to stop TrypLE™ Express action).
- Centrifuge cell suspension at **200 x *g*** for **3 minutes** at room temperature.
- Carefully remove the supernatant, (leaving a small amount of medium to ensure the cell pellet is not disturbed) and resuspend the cell pellet in **1 mL** of pre-warmed, **37°C**, **Melanocyte Proliferation Medium**.
- Perform a cell count to determine the number of viable cells and ensure optimal seeding density.
- Seed the cells on fibronectin-coated culture vessels at the recommended seeding density of **20,000 viable cells/cm²**. Use **2 mL** of **Melanocyte Proliferation Medium** per 10 cm^2 of culture surface.
- To ensure an even plating of **Human iPSC-Derived Melanocytes** gently rock the culture vessel back and forth and side to side twice.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator **overnight**.
- Every other day, conduct a full medium change, use **2 mL** of **Melanocyte Proliferation Medium** per 10 cm^2 of culture surface. For weekends, use **3 mL** **Melanocyte Proliferation Medium** per 10 cm^2 of culture surface.

Endpoint Assays

Once the desired number of **Human iPSC-Derived Melanocytes** has been generated the **Human iPSC-Derived Melanocytes** can cultured in **Melanocyte Growth Medium** to reduce proliferation and for a more elongated, spindle morphology.

The **Melanocyte Growth Medium** should be changed every other day.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
International phone **+44-1223-751-051**
US phone **+1-800-678-AXOL (2965)**

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Address

Axol Bioscience Limited | Suite 3 | The Science Village |
Chesterford Research Park | Little Chesterford | Cambridgeshire | CB10 1XL

International phone

+44-1223-751-051

US phone

+1-800-678-AXOL (2965)

Email

support@axolbio.com

Web

www.axolbio.com