Human iPSC-Derived Sebocytes

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0530	Human iPSC-Derived Sebocytes (Caucasian)	≥2 million cells/vial	N/A	Liquid Nitrogen	N/A	N/A
ax0531	Human iPSC-Derived Sebocytes (African)	≥2 million cells/vial	N/A	Liquid Nitrogen	N/A	N/A
ax0532	Human iPSC-Derived Sebocytes (Asian)	≥2 million cells/vial	N/A	Liquid Nitrogen	N/A	N/A
ax0538	Amplification Supplement	100 µL	1000x	Store at or below -20°C	Overnight at 4ºC	aliquot and store at -20ºC or maintain at room temperature for a maximum of 5 days
ax0539	Maturation Supplement	100 µL	1000x	Store at or below -20°C	Overnight at 4ºC	aliquot and store at -20ºC or maintain at room temperature for a maximum of 5 days
ax0048	Recombinant Human EGF	100 µg lyophilized Powder	N/A	-20ºC	N/A	Reconstituted protein should be used immediately or stored in working aliquots at -20ºC

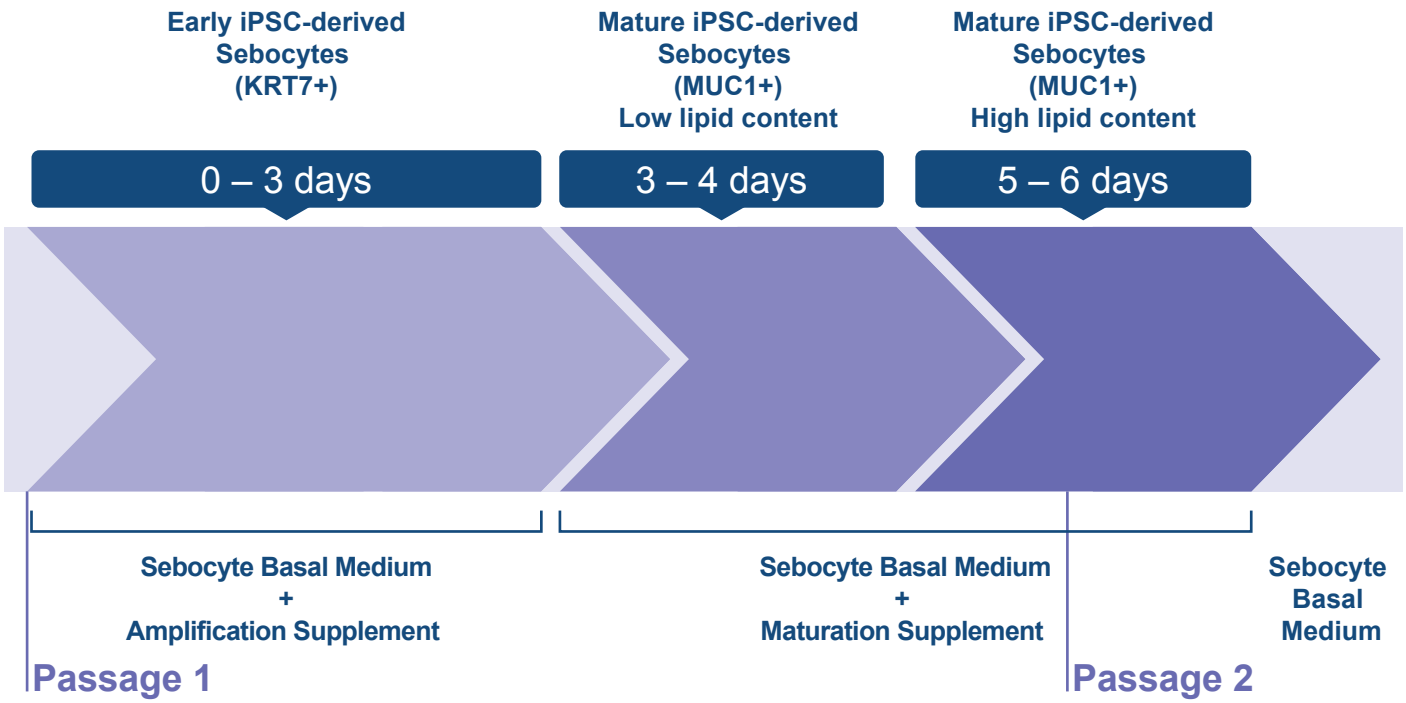
Additional Reagents		
Product Name	**Supplier**	**Product Code**
DMEM, high glucose, GlutaMAX™ Supplement	Thermo Fisher Scientific (Gibco)	61965026
Ham's F-12 Nutrient Mix	Thermo Fisher Scientific (Gibco)	21765029
Fetal Bovine Serum	Sigma-Aldrich	F7524
Insulin recombinant human	Sigma-Aldrich	I2643
Hydrocortisone	Sigma-Aldrich	H0888
Cholera Toxin from *Vibrio cholerae*	Sigma-Aldrich	C8052
Adenine	Merck Millipore	1152
Fibronectin	Sigma	F1141
TrypLE™ Express	Thermo Fisher	12605

Axol's **Human iPSC-Derived Sebocytes** are supplied as KRT7+ve proliferative cells. They can be used as models with a low lipid content (Method 1 using **Amplification Supplement**) or matured with a high lipid content (Method 2 using **Maturation Supplement**) using specific culture supplements.

Overview of protocol

If your research requires sebocytes with low lipid content, use “Method 1”.

If your research requires sebocytes with high lipid content, use “Method 2”.



Preparation of Reagents

Sebocyte Basal Medium

- Prepare Sebocyte Basal Medium by adding together the components in the table and filter sterilize the medium with a 0.22µm filter. Sebocyte Basal Medium can be stored at 4ºC for 1 month.

Medium Components	Final Concentration
DMEM, high glucose, GlutaMAX™ Supplement : Ham's F-12 Nutrient Mix	3:1 (vol:vol)
Fetal Bovine Serum	2.5%
Recombinant Human Insulin	10 ng/mL
Recombinant Human EGF	3 ng/mL
Hydrocortisone	45.2 ng/mL
Cholera Toxin from Vibrio cholerae	10^{-10}M
Adenine	24 µg/mL

Amplification Supplement

- Upon receipt, store **Amplification Supplement** at -20ºC. After thawing, supplements can be aliquoted and frozen, but should only be freeze/thawed once. Alternatively, **Amplification Supplement** can be maintained at room temperature for a maximum of 5 days.

Maturation Supplement

- Upon receipt, store **Maturation Supplement** at -20ºC. After thawing, supplements can be aliquoted and frozen, but should only be freeze/thawed once. Alternatively, **Maturation Supplement** can be maintained at room temperature for a maximum of 5 days.

Method 1 – Culture of Human iPSC-Derived Sebocytes with a low lipid content

Coating

- We recommend coating the tissue culture plates with fibronectin diluted to 1/100 in 1x PBS (use 0.1 mL per cm^2 cell culture surface). Incubate for at least **2 hours** in a **37ºC** incubator.
- Before use, remove fibronectin coating solution.

Thawing and Plating

- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **Sebocyte Basal Medium** warm to **37ºC** 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 **37ºC**, water bath.
- Quickly thaw the vial of cells in a **37ºC** 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 Sebocyte Basal Medium**.
- Centrifuge cell suspension at **250 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 **2 mL** of pre-warmed, **37ºC**, **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement**.
- 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 **25,000 viable cells/cm^2**. Use **2 mL** of **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement** per 10 cm^2 of culture surface.
- To ensure an even plating of **Human iPSC-Derived Sebocytes** 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.

Note:

One day after thawing, a significant amount of floating cells might be observed: this corresponds to normal cell death caused by our cell purification process. At day 5 of culture, **Human iPSC-Derived Sebocytes** lipid droplets accumulate in the cytoplasm, while lipid-filled cells detach from the culture plate and start holocrine secretion

Maintenance

- Change **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement** every other day using 2 mL/10 cm^2 culture surface (add 3 mL/10 cm^2 culture surface on weekends).
- Culture the **Human iPSC-Derived Sebocytes** in **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement** for at least 3 days after plating before using for assays.

Method 2 – Culture of Human iPSC-Derived Sebocytes with a high lipid content

Coating

- We recommend coating the tissue culture plates with fibronectin diluted to 1/100 in 1x PBS (use 0.1 mL per cm^2 cell culture surface). Incubate for at least **2 hours** in a **37ºC** incubator.
- Before use, remove fibronectin coating solution.

Thawing, Plating and Maintenance

- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **Sebocyte Basal Medium** warm to **37ºC** 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 **37ºC**, water bath.
- Quickly thaw the vial of cells in a **37ºC** 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 Sebocyte Basal Medium**.
- Centrifuge cell suspension at **250 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 **2 mL** of pre-warmed, **37ºC**, **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement**.
- 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 **25,000 viable cells/cm^2**. Use **2 mL** of **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement** per 10 cm^2 of culture surface.
- To ensure an even plating of **Human iPSC-Derived Sebocytes** gently rock the culture vessel back and forth and side to side twice.
- Incubate the cells at **37ºC**, **5% CO_2** in a humidified incubator.
- Culture the **Human iPSC-Derived Sebocytes** in **Sebocyte Basal Medium** supplemented with **1:1000 Amplification Supplement** for at least 3 days. Change the medium every other day using 2 mL/10 cm^2 culture surface (add 3 mL/10 cm^2 culture surface on weekends).
- Switch to **Sebocyte Basal Medium** supplemented with **1:1000 Maturation Supplement** for the next 2 days until cells reach 90% confluency and can be further passaged.

Note:

One day after thawing, a significant amount of floating cells might be observed: this corresponds to normal cell death caused by our cell purification process. At day 5 of culture, **Human iPSC-Derived Sebocytes** lipid droplets accumulate in the cytoplasm, while lipid-filled cells detach from the culture plate and start holocrine secretion.

Passaging

Human iPSC-Derived Sebocytes can be passaged once after thawing. Passaging of **Human iPSC-Derived Sebocytes** should be performed when the cells reach 90% confluency (usually 5-6 days after plating).

- Prepare culture vessels by coating with fibronectin at least 2 hours before thawing the **Human iPSC-Derived Sebocytes**.
- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **Sebocyte Basal Medium** and warm to **37ºC** prior to use.
- Pre-warm TrypLE™ Express to **37ºC**.
- 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 **Sebocyte Basal Medium** (anticipate at least a 1/3 dilution ratio to stop TrypLE™ Express action).
- Centrifuge cell suspension at **250 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, **37ºC**, **Sebocyte Basal Medium** (without supplements).
- 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 **25,000 viable cells/cm^2**. Use **2 mL** of **Sebocyte Basal Medium** (without supplements) per 10 cm^2 of culture surface.
- To ensure an even plating of **Human iPSC-Derived Sebocytes** 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.
- Change **Sebocyte Basal Medium** (without supplements) every other day using 2 mL/10 cm^2 culture surface (add 3 mL/10 cm^2 culture surface on weekends).
- Cells can be assayed as early as 24 hours after passage.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
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Notes

Notes

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