Human iPSC-Derived Retinal Pigment Epithelial Cells

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0540	Human iPSC-Derived Retinal Pigment Epithelial Cells (passage 2)	≥2 million cells/vial	N/A	Liquid Nitrogen	N/A	N/A

Additional Reagents		
Product Name	**Supplier**	**Product Code**
DMEM, high glucose	Gibco (Thermo Fisher Scientific)	11965092
Ham's F-12 Nutrient Mix	Gibco (Thermo Fisher Scientific)	11765054
B-27™ Supplement (50x)	Gibco (Thermo Fisher Scientific)	17504044
Antibiotic-Antimycotic (100x)	Gibco (Thermo Fisher Scientific)	15240062
Matrigel™ Basement Membrane Matrix Growth Factor Reduced	Corning Life Science	354230
Accumax™ Solution	Sigma-Aldrich	A7089

These reagents must be added fresh for each aliquot of medium.

Preparation of Reagents

Matrigel™ Matrix

- Upon receipt, aliquot and store **Matrigel™** at **20°C**, according to manufacturer's protocol.
- Coat tissue culture plates **6 hours** or **overnight** before thawing **Human iPSC-Derived Retinal Pigment Epithelial Cells**.

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RPE Cell Culture Medium

- Prepare **RPE Cell Culture Medium** by adding the following reagents:
- **RPE Cell Culture Medium** can be stored at 4°C for 1 month.

Reagent	Percentage	100 mL	500 mL
DMEM, high glucose	70%	70 mL	350 mL
Ham's F-12 Nutrient Mix	30%	30 mL	150 mL
B-27™ Supplement (50x)	2%	2 mL	10 mL
Antibiotic-Antimycotic (100x)	1%	1 mL	5 mL

Culture of Human iPSC-Derived Retinal Pigment Epithelial Cells

Coating

- We recommend coating the tissue culture plates at a final density of **8-10 µg/cm²** and incubate for **6 hours** (optimal is overnight) at **37°C** before thawing **Human iPSC-Derived Retinal Pigment Epithelial Cells**.
- Unused **Matrigel™**-coated plates can be stored at 4°C for a maximum of 5 days.
- DO NOT USE if **Matrigel™** coating has dried.

Thawing and Plating

- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **RPE Cell Culture 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 2 mL **RPE Cell Culture Medium**.
- Centrifuge cell suspension at **150 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**, **RPE Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells and ensure optimal seeding density.

Note:

Because of **Human iPSC-Derived Retinal Pigment Epithelial Cell** pigmentation, image-based trypan blue viability determination may be skewed towards lower percentages. For best results, use a fluorescent-based approach such as propidium iodide exclusion method.

- Seed the cells on **Matrigel™**-coated culture vessels at the recommended seeding density of **100,000 viable cells/cm²**. Use **3 mL** of **RPE Cell Culture Medium** per 10 cm² of culture surface.
- To ensure an even plating of **Human iPSC-Derived Retinal Pigment Epithelial Cells** 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**.

Maintenance

- Every day, conduct a full medium change, use **3 mL** of **RPE Cell Culture Medium** per 10 cm^2 of culture surface.
- For weekends, use **4 mL** **RPE Cell Culture Medium** per 10 cm^2 of culture surface on Saturday (no need to feed on Sunday).
- Within 15 days of plating at the recommended seeding density, **100,000 cell/cm^2**, the **Human iPSC-Derived Retinal Pigment Epithelial Cells** will acquire their characteristic polygonal morphology.
- After one month, pigmentation is visible to the naked eye (against a white background) and swollen fluid filled domes should appear. These areas confirm that the **Human iPSC-Derived Retinal Pigment Epithelial Cells** are functional and transport fluid from apical to basal sides.

Note:

Once the **Human iPSC-Derived Retinal Pigment Epithelial Cells** establish a confluent monolayer, they will quickly consume the nutrients in the culture medium, and produce metabolic waste which will turn the medium yellowish. Feeding everyday ensures the best results when culture is performed on regular flat surface (such as plates or flasks). **Human iPSC-Derived Retinal Pigment Epithelial Cells** cultured on Transwell™ insert, then medium feeding can be done every other day. Fluid filled domes will not appear when culturing **Human iPSC-Derived Retinal Pigment Epithelial Cells** on Transwell™ inserts.

Passaging

Passaging of **Human iPSC-Derived Retinal Pigment Epithelial Cells** can be performed every other week, when the cells have acquired their polygonal morphology and are lightly pigmented (usually only observable in the cell pellet during passaging).

- Prepare a sufficient volume (dependent on the culture vessel format for plating) of **RPE Cell Culture Medium** and warm to **37°C** prior to use.
- Remove culture medium and add **1 mL** Accumax for each 10 cm^2 of culture surface.
- Incubate at **37°C**, **5% CO_2** for **15-20 minutes**. Regularly check the cells and proceed to the next step when all the cells look rounded.
- Thoroughly flush the **Human iPSC-Derived Retinal Pigment Epithelial Cells** layer using the Accumax already in the dish. If cells do not readily detach, incubate at **37°C** for additional 5 minutes.
- Transfer the cells to a 15 mL sterile conical tube containing **2 mL** **RPE Cell Culture Medium** for each 1 mL of Accumax added.
- Centrifuge cell suspension at **150 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**, **RPE Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells and ensure optimal seeding density.

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- To ensure an even plating of **Human iPSC-Derived Retinal Pigment Epithelial Cells** 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 day, conduct a full medium change, use **3 mL** of **RPE Cell Culture Medium** per 10 cm^2 of culture surface.
- For weekends, use **4 mL** **RPE Cell Culture Medium** per 10 cm^2 of culture surface on Saturday (no need to feed on Sunday).

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