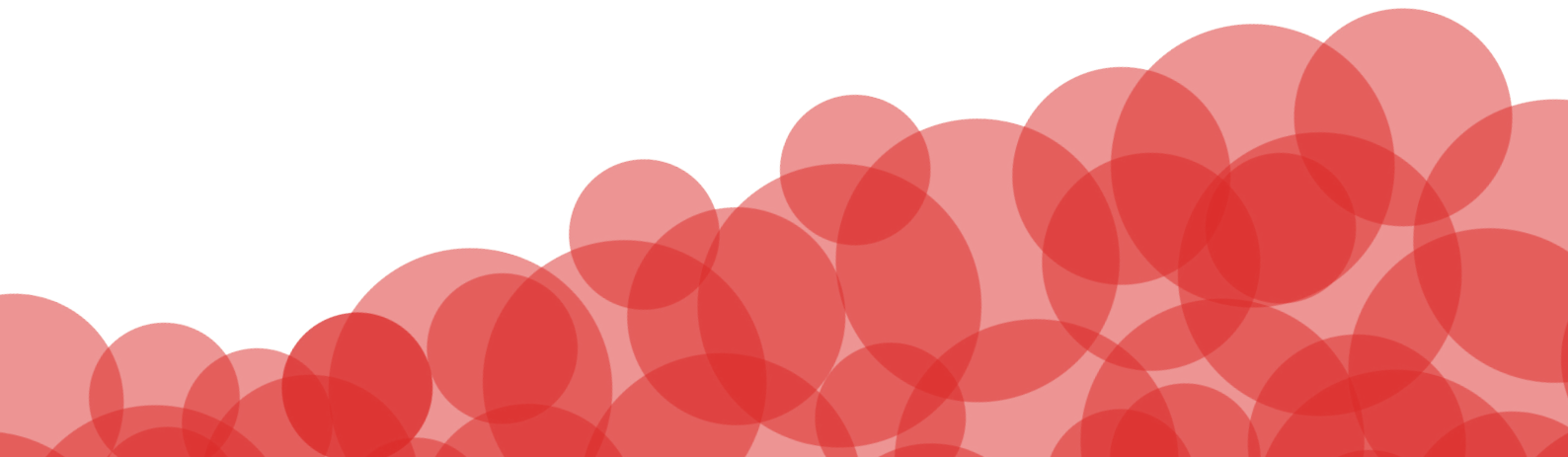
Human iPSC-Derived Endothelial Colony Forming Cells



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Human iPSC-Derived Endothelial Colony Forming Cells

Catalog. No.	Product Name	Product Quantity	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax2015	Human iPSC-Derived Endothelial Colony Forming Cells (Male)	1 million cells/vial	Liquid Nitrogen	Follow protocol	N/A
ax2019	Human iPSC-Derived Endothelial Colony Forming Cells (Female)	1 million cells/vial	Liquid Nitrogen	Follow protocol	N/A
ax2030	Endothelial Colony Forming Cell Culture Medium	500 mL	Aliquot and store at -80°C for up to 6 months	Thaw at 4°C or at room temperature	Store at 4°C for up to 1 week
ax0049	Fibronectin Coating Solution	1 mL	Aliquot and store at -80°C for up to 3 months	Thaw at 4°C	Once diltued, use immediately
ax0044	Unlock	25 mL	Aliquot & store at -80°C for up to 6 months	Thaw at 4°C	Store at 4°C for up to 1 week
ax2215	Human iPSC-Derived Endothelial Colony Forming Cell Kit (Male)	Kit Components • 1 million cells • Endothelial Colony Forming Cell Culture Medium • Fibronectin Coating Solution • Unlock	See above for component details	See above for component details	See above for component details
ax2219	Human iPSC-Derived Endothelial Colony Forming Cell Kit (Female)	Kit Components • 1 million cells • Endothelial Colony Forming Cell Culture Medium • Fibronectin Coating Solution • Unlock	See above for component details	See above for component details	See above for component details

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: **Fibronectin**
- Recommended cell culture medium: **Endothelial Colony Forming Cell Culture Medium**
- Recommended seeding density for assay: **10,000 viable cells/cm^2**
- Recommended centrifugation speed: **300 x *g* for 5 minutes**

Important!
Axol Endothelial Colony Forming Cell Culture Medium

DOES NOT contain antibiotics or antifungal agents.

Axol Bioscience does not recommend the use of antimicrobial agents such as penicillin, streptomycin and amphotericin. Antimicrobial agents should not be necessary if proper aseptic technique is adopted.

Coating

- Dilute the stock **Fibronectin Coating Solution** **1:100** in sterile water to make 1x working solution e.g. **100 µL** in **10 mL**.
- On the day prior to thawing the cells, coat the surface of your culture vessel with the **Fibronectin** 1x working solution. We recommend coating at a volume of **200 µL per cm²** however, please optimize for your experiments.
- Incubate the culture vessel **overnight** at **37°C** in a humidified incubator.

Thawing and Plating

- Having coated the culture vessel the day before, proceed with thawing the cells.
- 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. Remove the vial before the last bit of ice has melted, after ~1-2 minutes.
- Wipe the outside of the vial with 70% ethanol.
- Gently resuspend the cells and transfer to a 15 mL sterile conical tube.
- Slowly add **10 mL** of pre-warmed, **37°C**, **Endothelial Colony Forming Cell Culture Medium**.
- Rinse the cryovial with **1 mL** of **Endothelial Colony Forming Cell Culture Medium** to ensure all of the cells are transferred.
- Centrifuge the cells at **300 x *g*** for **5 minutes**. Carefully remove the supernatant and resuspend the cell pellet in **1-2 mL** of pre-warmed, **37°C**, **Endothelial Colony Forming Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, **37°C**, **Endothelial Colony Forming Cell Culture Medium**.
- Seed cells into the **pre-coated** culture vessel at the recommended seeding density of **10,000 viable cells/cm²**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.
- Frequency of media changes: **Every 2 days**

Passaging

- Passage when the culture reaches: **80-90% confluent**
- Recommended passaging reagent: **Unlock**

- On the day prior to passaging the cells, coat the culture vessel with **Fibronectin** 1x working solution and incubate **overnight** at **37°C** in a humidified incubator.
- Remove all spent culture medium from the cell culture vessels.
- Gently rinse the surface of the cell layer once with PBS, **2 mL** of PBS per **10 cm²** culture surface area. Discard the PBS.
- Add **1 mL per 10 cm²** of culture surface area of cold/room temperature **Unlock** passaging reagent. Evenly distribute it over the entire cell layer.
- 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, dilute out the passaging reagent with **four volumes** pre-warmed, **37°C**, **Endothelial Colony Forming Cell Culture Medium**. For example, if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction.
- Transfer the cell suspension to a sterile conical tube.
- Centrifuge the cells at **300 x *g*** for **5 minutes**.
- Carefully remove the supernatant and resuspend the cell pellet in **1-2 mL** of pre-warmed, **37°C**, **Endothelial Colony Forming Cell Culture Medium**.
- Perform a cell count to determine the number of viable cells.
- Dilute the cells into the required volume of pre-warmed, **37°C**, **Endothelial Colony Forming Cell Culture Medium**.
- Seed cells into the **pre-coated** culture vessel at the recommended seeding density of **10,000 viable cells/cm²**.
- Incubate the cells at **37°C**, **5% CO_2** 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?
Contact Axol Technical Support at **support@axolbio.com**
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Notes

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