



Human iPSC-Derived Ventricular Cardiomyocytes

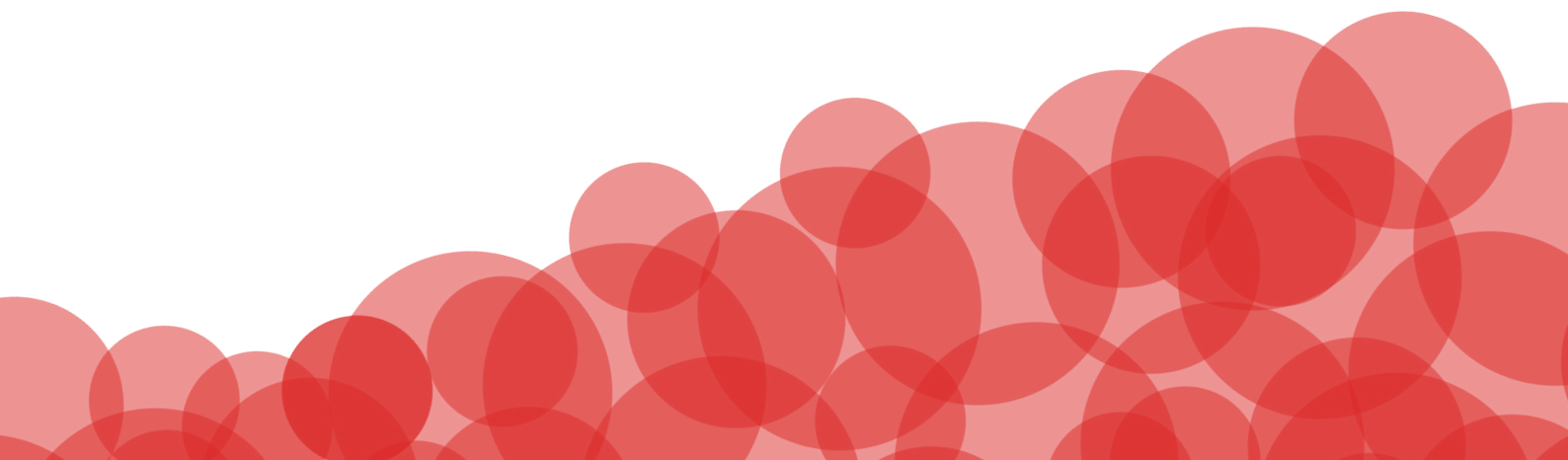


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Culture of Human iPSC-Derived Ventricular Cardiomyocytes

Catalog. No.	Product Name	Quantity	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax2505	Human iPSC-Derived Ventricular Cardiomyocytes - Male	1 million cells/vial	N/A	Liquid Nitrogen	Follow Protocol	N/A
ax2530-500	Cardiomyocyte Maintenance Medium	1 x 500 mL Basal Medium 1 x 10 mL Supplement	1x 1x	Store the Basal Medium at 4°C and the Supplement at -80°C	Thaw the Supplement overnight at 4°C	Once thawed store at 4°C. If required, the medium can be aliquoted and stored at -80°C for later use.
ax0044	Unlock	25 mL	1x	Aliquot and store at -80°C. Store for up to 12 months at -80°C.	Thaw aliquot overnight (at 4°C)	Use within 1 week once thawed.

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis.

Recommendations

- Recommended culture vessel coating: **Matrigel™ or Fibronectin**
- Recommended cell culture medium: **Cardiomyocyte Maintenance Medium**
- Recommended seeding density for assay: **50,000-200,000 viable cells/cm²**
- Recommended centrifugation speed: **200 x g for 5 minutes**
- Recommended days in culture before assay: **7-10 days**

Important! Cardiomyocyte Maintenance Medium = Basal medium + Supplement

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.

Preparing Cardiomyocyte Maintenance Medium

Cardiomyocyte Maintenance Medium

- Upon receipt, store the **Cardiomyocyte Maintenance Basal Medium** at **4°C** and the **Supplement** at **-80°C**.
- Add the **Supplement** to the **Cardiomyocyte Maintenance Basal Medium**. For long-term storage, prepare aliquots of **Cardiomyocyte Maintenance Medium** and store at **-80°C**. The **Cardiomyocyte Maintenance Medium** is then stable for 6 months from the date of manufacture.

Plating Medium

- When ready to use, thaw an aliquot of **Cardiomyocyte Maintenance Medium** overnight at **4°C** in the dark.
- Take an aliquot of **Cardiomyocyte Maintenance Medium** and add **10% Fetal Bovine Serum (FBS)** to make up the **Plating Medium**. For example, combine 18 mL of **Cardiomyocyte Maintenance Medium** and 2 mL of FBS.
- Before use, pre-warm an aliquot of **Plating Medium** at **37°C**.

For a serum-free system **Matrigel™** is the recommended coating reagent and can be used without the plating medium

Unlock

- Upon receipt, aliquot and store **Unlock** at or below **-80°C**. Stored at **-80°C**, **Unlock** is stable for 12 months from the date of manufacture.
- When ready to use, thaw an aliquot overnight at **4°C** and use either at **4°C** or **room temperature**. **Do not pre-warm**.

Coating the Culture Vessel

Matrigel™ Coating

- Calculate the total surface area that requires coating.
- Dilute the **Matrigel™** in sterile medium (such as DMEM/RPMI) at a 1:100-1:200 dilution. This dilution should be optimized for individual experiments.
- Coat the surface of your culture vessel with the diluted **Matrigel™** solution. We recommend coating at a volume of **200 μL per cm^2** , however, please optimize for your experiments.
- Incubate for **2 hours** at **37°C**.
- Remove the diluted **Matrigel™** from the culture vessel before plating cells.

Fibronectin Coating

- Calculate the total surface area that requires coating.
- Dilute the **Fibronectin** in sterile distilled water to **10 $\mu\text{g}/\text{mL}$** . This dilution should be optimized for individual experiments.
- Coat the surface of your culture vessel with the **Fibronectin (10 $\mu\text{g}/\text{mL}$)** solution. We recommend coating at a volume of **200 μL per cm^2** , however, please optimize for your experiments.
- Incubate for **4 hours** at **37°C**.
- Remove the diluted **Fibronectin** from the culture vessel before plating cells.

Thawing and Plating Human iPSC-Derived Ventricular Cardiomyocytes

- On the day of thawing **Human iPSC-Derived Ventricular Cardiomyocytes cells**, prepare the **Cardiomyocyte Maintenance Medium** and **Plating Medium**.
- Prepare culture vessels with the desired coating matrix.
- Pre-warm all media and vessels to **37°C** before use.
- To thaw cells – transfer the cells from liquid nitrogen storage by carrying cells buried in dry ice to a water bath. Remove the cells from dry ice and transfer them immediately 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 two thirds 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 cabinet, spraying it thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing it in the hood.
- Once thawed, use a P1000 pipette to immediately transfer the cells drop-wise into a 15 mL sterile conical tube containing **10 mL** of pre-warmed **Plating Medium**. Gently wash the vial with **1 mL** of **Plating Medium**. Transfer this to the 15 mL sterile conical tube containing the cells.

Do not mix the cells vigorously. Avoid generating bubbles.

- Centrifuge cells at **200 x g** for **5 minutes** at room temperature.
- Aspirate and discard the supernatant carefully. Resuspend the cell pellet in **1 mL** of **Plating Medium**.
- Gently resuspend the cells until they are in a single cell suspension.
- **Perform a cell count to ensure optimal seeding density.**
- Remove **10 µL** of cell suspension and mix it with **10 µL** of trypan blue solution. Count the cells.
- Remove the coating reagent from the culture vessel before plating the resuspended cells.
- Plate the resuspended cells drop-wise and evenly at a seeding density no less than **50,000 cells/cm²** on the pre-coated culture vessel.
- To ensure an even plating of cardiomyocytes, gently rock the culture vessel back and forth and side to side several times.

Seeding density is application dependent and needs to be optimized.

- Incubate the cells at **37°C, 5% CO₂**.
- The day after plating, replace the culture medium with fresh, pre-warmed (**37°C**) **Cardiomyocyte Maintenance Medium** (without 10% FBS) to remove any dead cells/debris and to promote proliferation.

Maintenance and Maturation of Human iPSC-Derived Ventricular Cardiomyocytes

- **Every 2 days** remove half the medium and replace with the same volume of fresh, pre-warmed (**37°C**) **Cardiomyocyte Maintenance Medium**.
- After 7 days in culture, the **Human iPSC-Derived Ventricular Cardiomyocytes** should beat spontaneously (this can occur within 72 hours).
- After 7-10 days in culture, **Human iPSC-Derived Ventricular Cardiomyocytes** will be ready for experiment assays. **Human iPSC-Derived Ventricular Cardiomyocytes** can be cultured for longer depending on assay requirements.

Re-plating Human iPSC-Derived Ventricular Cardiomyocytes

While **Human iPSC-Derived Ventricular Cardiomyocytes** retain some proliferation capacity, we do not encourage passaging cells for expansion as the cells will exit the cell cycle, leading to cell loss. However, if you need to transfer the cells from one culture vessel to one of a different format, please follow our instructions below.

Re-plating should be conducted within 3 days of initial seeding.

- Prepare culture vessels with the desired coating matrix.
- Prepare **Plating Medium** by adding **10% FBS** to **Cardiomyocyte Maintenance Medium**.
- Pre-warm all media and culture vessels to **37°C** before use.
- Thaw **Unlock** overnight at **4°C** before use and store at **4°C**.
- Discard the spent medium from the culture vessel.
- Gently rinse the surface of the cell layer once with the Dulbecco's-PBS (1x) (D-PBS, without calcium or magnesium, **2 mL D-PBS per 10 cm² culture surface area**).
- Discard the D-PBS.
- To detach the cells, add **1 mL** of cold/room temperature **Unlock** per 10 cm² culture surface area. Evenly distribute it over the entire cell layer. Incubate the cells for **5 minutes** at **37°C** in the incubator.
- Use a P1000 pipette to transfer the cells drop-wise into a 15 mL sterile conical tube containing **four volumes** of pre-warmed **Plating Medium**. (e.g. if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction). Gently pipette up and down a few times to disperse the medium.
- Centrifuge cells at **200 x g** for **5 minutes** at room temperature.
- Aspirate and discard the supernatant carefully. Gently resuspend the cell pellet in **1 mL** of **Plating Medium** until the cells are in a homogeneous single cell suspension.
- **Perform a cell count to ensure optimal seeding density.**
- Remove coating reagent from the culture vessel before plating resuspended cells.

- Plate the resuspended cells drop-wise and evenly at a seeding density no less than **50,000 cells/cm²** on the pre-coated culture vessel.
- To ensure an even plating of **Human iPSC-Derived Ventricular Cardiomyocytes** gently rock the culture vessel back and forth and side to side several times.
- Incubate the cells at **37°C, 5% CO₂**.
- The day after plating, replace the medium with fresh, pre-warmed (**37°C**) **Cardiomyocyte Maintenance Medium** (without 10% FBS), to remove any dead cells/debris and to promote proliferation.
- **Every 2 days** remove half the medium and replace with the same volume of fresh, pre-warmed (**37°C**) **Cardiomyocyte Maintenance Medium**.

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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