axol

Human iPSC-Derived Ventricular Cardiomyocytes

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Product Information

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
ax2520	Human iPSC-Derived Ventricular Cardiomyocytes - Male	1 million cells/vial	N/A	Liquid Nitrogen	Follow Protocol	N/A
ax2502	Human iPSC-Derived Ventricular Cardiomyocytes - Female	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 4ºC and the Supplement at -80ºC	Thaw the Supplement overnight at 4ºC	Once thawed store at 4ºC. If required, the medium can be aliquoted and stored at -80ºC for later use.
ax0049	Fibronectin Coating Solution	1 mL	100x	Aliquot and store at -80ºC for up to 3 months	Thaw at 4ºC	Once diluted, use immediately
ax2500	Human iPSC-Derived Ventricular Cardiomyocyte Kit - Male	Kit Components • 1 million cells • Cardiomyocyte Maintenance Medium • Fibronectin Coating Solution	See above for component details	See above for component details.	See above for component details.	See above for component details.

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Y-27632 dihydrochloride (ROCK inhibitor)	Focus Biomolecules	10-2301
Fetal bovine serum (FBS)-EU Approved heat inactivated	Sigma Aldrich	F9665-500ML

Individual experimental results may vary depending on the supplier and batch of FBS used.

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 **4ºC** and the **Supplement** at **-80ºC**.
- Add the **Supplement** to the **Cardiomyocyte Maintenance Basal Medium**. For long-term storage, prepare aliquots of **Cardiomyocyte Maintenance Medium** and store at **-80ºC**. 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 **4ºC** in the dark.
- Take an aliquot of **Cardiomyocyte Maintenance Medium** and add 10% fetal bovine serum (FBS) and Y-27632 2HCl (ROCK inhibitor) to a final concentration of 10 µM to make up **Plating Medium**.

Plating Medium			
Supplement	**Stock Concentration**	**Final Concentration**	**50 mL Medium**
Y-27632 dihydrochloride (ROCK inhibitor)	10 mM	10 µM	50 µL
FBS	n/a	n/a	5 mL

- Before use, pre-warm an aliquot of **Plating Medium** at **37ºC**.

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 cm²**, however, please optimize for your experiments.
- Incubate for **2 hours** at **37ºC**.
- Remove the diluted **Matrigel™** from the culture vessel before plating cells.

Fibronectin Coating

- Calculate the total surface area that requires 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.
- 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 **37ºC** 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. Add a small volume of pre-warmed (**37°C**) **Plating Medium** to the culture vessel so that it does not dry out.
- Plate the resuspended cells drop-wise and evenly at a seeding density no less than **50,000 cells/cm^2** on the pre-coated culture vessel.
- Ensure that there is enough medium in the culture vessel to prevent drying and improper attachment. For example: include 2 mL total in a 6-well plate, 1 mL total in a 12-well plate and 500 µL in a 24-well plate..
- To ensure an even plating of cardiomyocytes, gently rock the culture vessel back and forth and side to side several times.

Please note that seeding density needs to be optimized by the user to suit their culture dish size, culture conditions and the final assay

- Incubate the cells at **37°C**, **5% CO_2**.
- The day after plating, replace the culture medium with fresh, pre-warmed (**37ºC**) **Cardiomyocyte Maintenance Medium** (without 10% FBS or Y-27632 2HCl (ROCK inhibitor)) to remove any dead cells/debris.

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 (**37ºC**) **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.

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