axoCells™

Human iPSC-Derived Atrial and Ventricular Cardiomyocytes

User Guide

Version 2

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This protocol and its associated products are for research use only.

Products are not for diagnostic or therapeutic use and are not to be administered to humans or animals.

Updates to protocol:

Part	Change	Rationale	Previous version	New version
Human Fibronectin	New part	Material no longer supplied	1ml of ax0049 at 100x stock, diluted for use to 1:100 solution	ax0050 diluted entirely in 20 ml D-PBS.
Human iPSC-Derived Atrial Cardiomyocyte Kit - Male	New kit	New kit		New atrial cardiomyocyte kit ax2510 added to document.

Product Information

Catalog No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax2508	Human iPSC-Derived Ventricular Cardiomyocytes (Male)	1.0 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax2518	Human iPSC-Derived Atrial Cardiomyocytes (Male)	1.0 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 50x	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 for 2 weeks. If required, the medium can be aliquoted and stored at -80°C for later use.
ax0050	Human Fibronectin	1 vial	N/A	Store at -80°C	Thaw at 4°C	Once diluted, use immediately
ax2500	Human iPSC-Derived Ventricular Cardiomyocyte Kit - Male	Kit Components: 1 million cells; Cardiomyocyte Maintenance Medium; Human Fibronectin	See above for component details			
ax2510	Human iPSC-Derived Atrial Cardiomyocyte Kit - Male	Kit Components: 1 million cells; Cardiomyocyte Maintenance Medium; Human Fibronectin	See above for component details			

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Y-27632 2HCl (ROCK inhibitor)	Focus Biomolecules	10-2301
Fetal bovine serum (FBS)-EU Approved heat inactivated	Sigma Adrich	F9665-500mL

Individual experimental results may vary depending on the supplier and batch of FBS used. For Japan, we recommend the use of heat-inactivated FBS (Netherland origin) #S-FBS-NL-025 from Serana.

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis. Expiry dates for Axol-supplied components are stated on the label. Consult the manufacturer's guidelines for expiry dates of any additional reagents.

Recommendations

- Recommended culture vessel: **Greiner 96 well, Part no. 655090**
- Recommended culture vessel coating: **Fibronectin**
- Recommended cell culture medium: **Cardiomyocyte Maintenance Medium**
- Recommended seeding density for assay: **100,000 – 200,000 cells/cm²**
- Recommended centrifugation speed: **200 x g for 5 minutes**
- Recommended days in culture before assay: **7 to 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 techniques are adopted.

Coating Cell Cultureware

Fibronectin Coating

- Cell cultureware can be coated on the day of use, or overnight prior to use.
- Calculate the total surface area that requires coating.
- Dilute the stock **Human Fibronectin** in 20 ml D-PBS (without magnesium and calcium) to make a 1x working solution.
- Coat the surface of your culture vessel with the **Fibronectin** 1x working solution. We recommend coating at a minimum volume of 100 µl per well of a 96 well plate. However, please optimize for your experiments.
- Incubate the culture vessel for a minimum of 4 hours or overnight at 37°C in a humidified incubator.
- Coated plates can be stored at 4°C for up to 2 weeks. Any excess coating cannot be stored and should be discarded.

Preparation of Reagents

Cardiomyocyte Maintenance Medium

- Upon receipt store **Cardiomyocyte Maintenance Basal Medium** at 4°C and **Supplement** at -80°C.
- Remove and discard 10mL of the basal medium and then add 10mL **Supplement** to the remaining 490mL **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 from the first expiry date of media/supplement (please refer to the Certificate of Analysis).

Plating Medium

- When ready to use, thaw an aliquot of **Cardiomyocyte Maintenance Medium** overnight in the dark at 4°C.
- 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 the **Plating Medium**. Complete plating media must be used fresh and cannot be stored once supplemented.
- Before use, pre-warm an aliquot of **Plating Medium** at 37°C.

Plating Medium			
Supplement	**Stock Concentration**	**Final Concentration**	**Final Volume in 45 mL of Medium**
Y-27632 dihydrochloride (ROCK inhibitor)	10 mM	10 µM	50 µl
Fetal bovine serum (FBS)	NA	10%	5 ml

Thawing and Plating of axoCells Atrial & Ventricular Cardiomyocytes

- On the day of thawing **axoCells Atrial or Ventricular Cardiomyocytes**, prepare the **Cardiomyocyte Maintenance Medium** and **Plating Medium** and pre warm before use.
- To thaw the cells, transfer the cells from liquid nitrogen storage by carrying cells buried in dry ice. 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, taking care not to completely submerge the vial (only up to two thirds should be placed in the water). Remove the vial before the last bit of ice has melted, ~ 2 minutes.
- Do not shake the vial whilst thawing.
- Take the vial of cells to a biological cabinet, spraying it thoroughly with 70% ethanol and wipe with an autoclaved paper towel before placing in the culture hood.
- Once thawed, use a P1000 pipette to immediately transfer the cells to a 15 ml sterile

conical tube.

- Using a P1000, wash the now empty cryovial with 1 ml of room temperature **Plating Medium**. Add the 1 ml of **Plating Medium** to the conical tube containing the Cardiomyocytes.
- **Important** – the **Plating Medium** should be added to the cells drop-wise whilst gently swirling the conical tube. This should take ~ 60 seconds to dispense the 1 ml of medium. This is a necessary step to prevent osmotic shock to the cells and improve post thaw viability.
- Using a 10 ml stripette, slowly add 8 ml of room temperature **Plating Medium** to conical tube containing the cells. This should take ~ 60 seconds to dispense the 8 ml of medium.
- Centrifuge the cells at 200 g for 5 minutes at room temperature.
- Aspirate and discard the supernatant, taking care not to disturb the cell pellet.
- Using a P1000 pipette, resuspend the pellet in 1 ml of warm (37°C) **Plating Medium**. Gently resuspend the cell pellet until a single cell suspension is obtained.
- 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.
- Using warm, 37°C **Plating Medium**, resuspend the cells to give the required plating density.
- Remove the fibronectin solution from the plate to be seeded, take care to work quickly so that the plate does not dry out.
- Plate the cells at a density of 100,000 – 200,000 cells/cm^2. Depending on application, this density may require optimization.

Please note that this is a recommended seeding density, and that density may need to be optimized by the user to suit their culture dish size, culture conditions and the final assay

- 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 500 µL in a 24-well plate and 100 µL in a 96-well plate.
- To ensure an even plating of cardiomyocytes, gently rock the culture vessel back and forth and side to side several times.
- Incubate the cells at 37°C, 5% CO_2.
- The day after plating, replace the culture medium with fresh, pre-warmed (37°C)

Cardiomyocyte Maintenance Medium (without 10% FBS or Y-27632 2HCl (ROCK inhibitor) to remove any dead cells/debris.

Maintenance of axoCells Atrial & Ventricular Cardiomyocytes

- Every 2 days remove the medium and replace with the same volume of fresh, pre-warmed (37°C) **Cardiomyocyte Maintenance Medium**.

- After 7 days in culture, the **axoCells Atrial or Ventricular Cardiomyocytes** should beat spontaneously (this can occur within 72 hours).

- After 7-10 days in culture, **Atrial or Ventricular Cardiomyocytes** will be ready for experimental assays. **Atrial or 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

Or

Call +44 (0) 131 651 9710