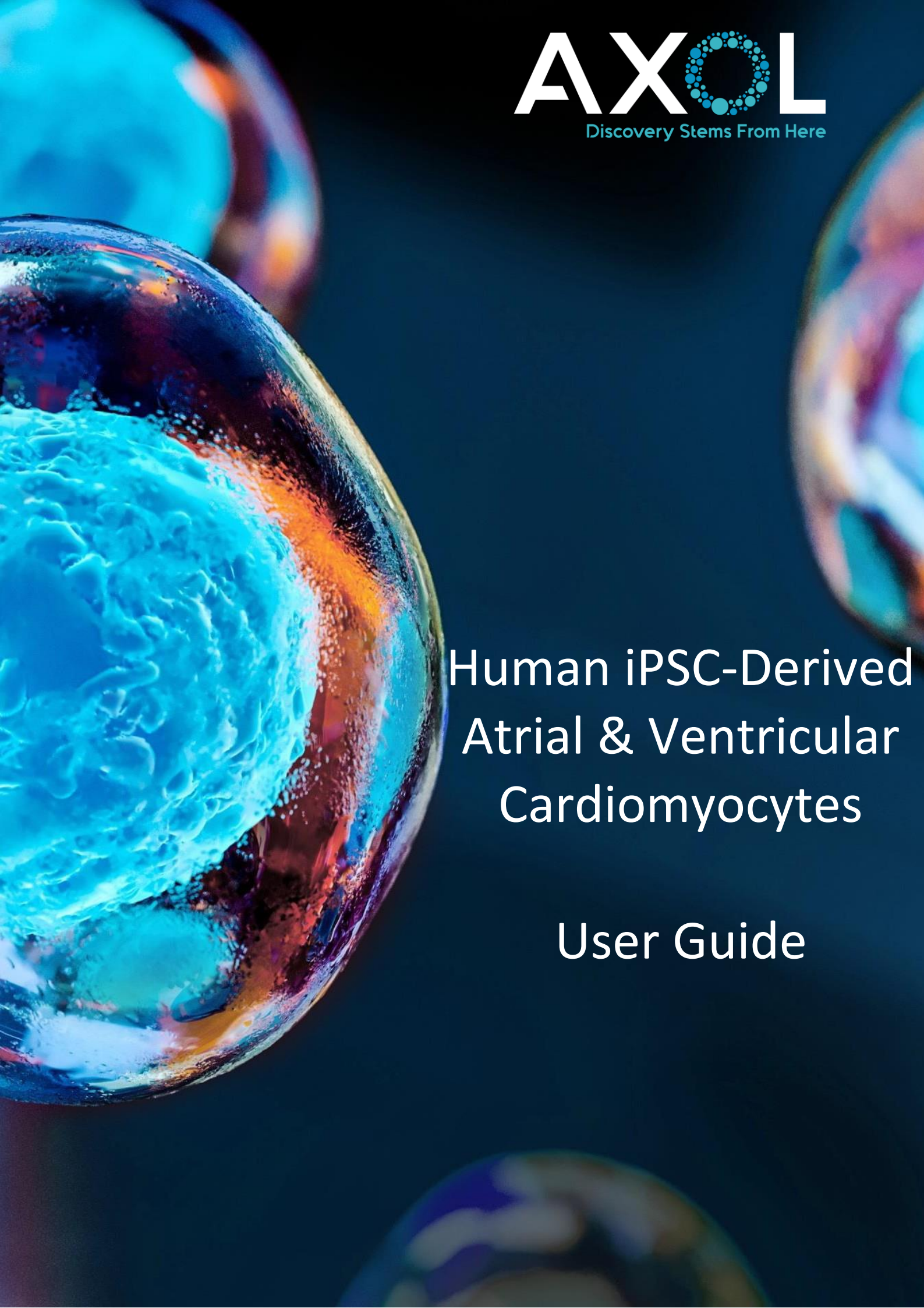

Human iPSC-Derived Atrial & Ventricular Cardiomyocytes

User Guide

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 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: **Greiner 96 well, Part no. 655090**
- Recommended culture vessel coating: **Fibronectin**
- Recommended cell culture medium: **Cardiomyocyte Maturation Medium**
- Recommended seeding density for assay: **100,000 – 200,000 cells/cm^2**
- 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.

Preparation of reagents

Cardiomyocyte Maintenance Medium

- Upon receipt store Cardiomyocyte Maintenance Medium at or below 4°C and 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 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.

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

- Before use, pre-warm an aliquot of Plating Medium at 37°C

Coating the Culture Vessel

Fibronectin Coating

- Calculate the total volume that is required for coating.
- Dilute the stock Fibronectin Coating Solution 1:100 in sterile water to make a 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 minimum volume of 100 µl per well of a 96 well plate. However, please optimize for your experiments.
- Incubate the culture vessel overnight at 37°C in a humidified incubator.

Thawing & Plating Human iPSC-Derived Atrial & Ventricular Cardiomyocytes

- On the day of thawing Human Derived Atrial or Ventricular Cardiomyocytes, prepare the Cardiomyocyte Maintenance Medium and Plating Medium.
- 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 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, 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 200 µL in a 96-well palte.
- 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% CO2.
- 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 of Human iPSC-Derived Atrial & Ventricular Cardiomyocytes

- Every 2 days remove 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 Atrial or Ventricular Cardiomyocytes should beat spontaneously (this can occur within 72 hours).
- After 7-10 days in culture, Human iPSC-Derived Atrial or Ventricular Cardiomyocytes will be ready for experimental assays. Human iPSC-Derived Atrial or Ventricular Cardiomyocytes can be cultured for longer depending on assay requirements.

Notes

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
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Or
call +44 (0) 1223 751051