AXOL

Discovery Stems From Here

Human iPSC-Derived Atrial Cardiomyocytes

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax2515	Human iPSC-Derived Atrial Cardiomyocytes (Healthy)	≥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 4°C	Once thawed, aliquot and store at 4ºC. If required, the medium can be aliquoted and stored at -80ºC for later use.
ax0049	Fibronectin Coating Solution	1 mL	1 mg/mL	Aliquot and store at -80°C for 3 months	Overnight at 4°C	Once diluted, use immediately.
ax68168 (5 mg)	Y-27632 2HCl (ROCK inhibitor)	5 mg Lyophilized Powder	N/A	Stable for 2 years at -20°C. Solutions in DMSO or methanol may be stored at -20°C for up to 3 months	N/A	Reconstituted protein should be used immediately or stored in working aliquots at -20°C

Additional Reagents		
Product Name	**Supplier**	**Product Code**
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.

Important! Axol Cell Culture Media

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.

Recommendations

- Recommended culture vessel coating: Matrigel™ or Fibronectin
- Recommended cell culture medium: Cardiomyocyte Maintenance Medium
- Recommended seeding density for assay: 100,000-150,000 viable cells/cm^2
- Recommended centrifugation speed: 300 x g for 5 minutes
- Recommended days in culture before assay: 7-10 days

Preparation of Reagents

Cardiomyocyte Maintenance Medium

- Upon receipt, aliquot and store Cardiomyocyte 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 -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**	**Volume to add in 50 mL**
Y-27632 2HCl (ROCK inhibitor) (ax68168 (5 mg))	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 cm^2, 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 mg/mL to a final concentration of 40 µg/mL in sterile water to make 1x working solution e.g. 400 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 cm^2 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.

Culture of Human iPSC-Derived Atrial Cardiomyocytes

Thawing and Plating

- On the day of thawing Human iPSC-Derived Atrial Cardiomyocytes, 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 the cells – transfer the vial of cells from storage by transporting the vial buried in dry ice. Remove the vial from dry ice and transfer it 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 2/3rd 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 safety cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial 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 300 x *g* for 5 minutes at room temperature.
- Carefully aspirate and discard the supernatant using a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in 1 mL of Plating Medium until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.
- 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 100,000-150,000 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.
- 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 of Human iPSC-Derived Atrial Cardiomyocytes

- Every 2 days conduct a full medium change with fresh, pre-warmed, 37ºC, Cardiomyocyte Maintenance Medium.
- After 7 days in culture, the Human iPSC-Derived Atrial Cardiomyocytes should beat spontaneously (this can occur within 72 hours).
- After 7-10 days in culture, Human iPSC-Derived Atrial Cardiomyocytes will be ready for experiment assays. Human iPSC-Derived Atrial Cardiomyocytes can be cultured for longer depending on assay requirements.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
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Or
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