



axoCells™ Human iPSC-Derived
Ventricular Cardiomyocytes
Advanced Maturation using the
MyoMax Maturation Medium Kit

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:

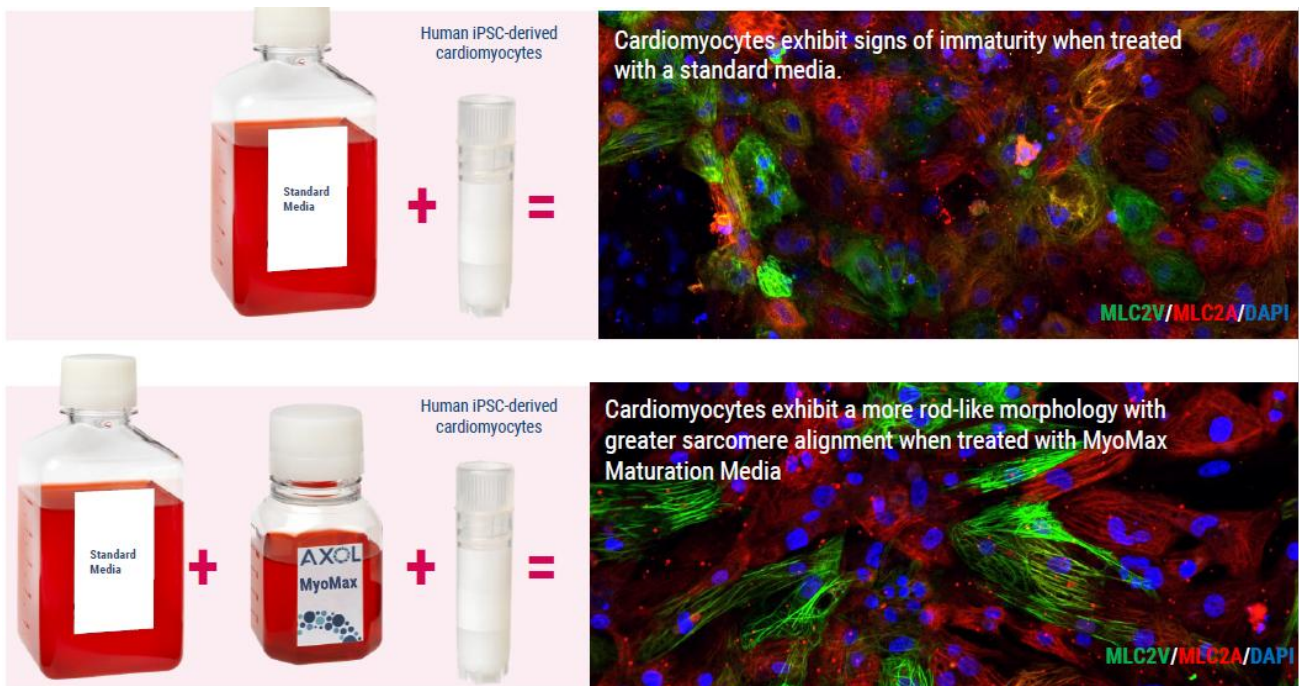
Part	Change	Rationale	Previous version	New version
All	Temporary removal of references to Atrials	Pending validation	Guidance for Atrial & Ventricular Cardiomyocytes	Ventricular Cardiomyocytes only
Product Overview	Inclusion of Product Overview	Provides product context and intended use	Absence of Product Overview	Inclusion of Product Overview
Recommendations	Addition of guidance on media re-gassing	Offers additional flexibility for end user	No guidance given	Included wording to allow re-gassing of media if pink

Product Overview

axoCells™ MyoMax Maturation Medium is a metabolic maturation medium designed to enhance the maturation of axoCells™ Ventricular Human iPSC-derived cardiomyocytes. It is designed for users aiming to generate more adult-like cardiomyocytes for applications such as improved drug response profiling, advanced phenotypic characterisation of *in vitro* disease models and/or greater structural integrity in 3D applications.

MyoMax™ Maturation Medium promotes maturation by shifting cellular metabolism from glycolysis towards fatty acid oxidation. After following at least seven days of the maturation protocol, cardiomyocytes typically demonstrate a more rod-shaped morphology, improved sarcomere alignment, a reduction in spontaneous beating, faster 2-point conduction velocity and upregulated expression of maturation markers such as *KCNJ2* & Connexin-43.

Due to the nature of this metabolic shift, MyoMax™ Maturation Medium should be introduced gradually, and its use is recommended with highly pure cardiomyocyte populations.



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
ax2558		5.0 million cells/vial				
ax2535-125	Cardiomyocyte Maintenance Medium-XF	1 x 125 ml Basal Medium 1 x 2.5 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. If required, the medium can be aliquoted and stored at -80°C for later use
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. If required, the medium can be aliquoted and stored at -80°C for later use
ax0050	Fibronectin Coating Solution	1 vial	N/A	Store at -80°C	Thaw at 4°C	Once diluted, use immediately
ax2550	MyoMax Maturation Medium Kit	1x 100 ml Maturation Basal Medium 1x 15 ml Maturation Supplement A 1x 9 ml Maturation Supplement B 1x 1 ml Maturation Supplement C	1x Basal Supplement A (5x) Supplement B (10x) Supplement C (1x)	Store the Basal Medium at 4°C and the Supplements at -20°C	Thaw the Supplements overnight at 4°C	Once thawed store at 4°C for up to one week

Additional Reagents			
Product Name	Supplier	Product Code	Preparation
Y-27632 2HCl (ROCK inhibitor)	Focus Biomolecules	10-2301	Follow manufacturer's instructions
Fetal bovine serum (FBS)-EU Approved heat inactivated	Sigma Aldrich	F9665-500ML	Follow manufacturer's instructions

Individual experimental results may vary depending on the supplier and batch of FBS used. For Japan, we recommend the use of heat-inactivated FBS (Netherlands 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

Culture vessel	Greiner 96 well, Part no. 655090
Culture vessel coating	Axol Human Fibronectin
Cell Culture Medium	Cardiomyocyte Maintenance Medium-XF or Cardiomyocyte Maintenance Medium
Seeding density for assay	180,000 – 220,000 cells/cm ²
Centrifugation speed (thawing)	200 x g for 5 minutes
Culture duration prior to assay	7 days

Important! Cardiomyocyte Maintenance Medium-XF = Basal Medium + Supplement DOES NOT contain antibiotics or antifungal agents.

Cardiomyocyte Maintenance Medium = Basal Medium + Supplement DOES NOT contain antibiotics or antifungal agents.

MyoMax Maturation Medium = Basal Medium + Supplements DOES NOT contain antibiotics or antifungal agents.

Complete MyoMax Maturation Medium can be stored at 4°C for up to one week. If the complete medium turns pink due to the phenol red pH indicator, the medium can be re-gassed in a 5% CO₂ incubator to equilibrate. Please note that this medium is buffered using sodium bicarbonate and not HEPES.

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.

Attention! MyoMax Maturation Medium is a metabolic maturation medium that rapidly shifts metabolism from glycolysis towards fatty acid oxidation. Cardiomyocytes must be gently transitioned into this medium to prevent excess cell death.

Coating Cell Culture Ware

Fibronectin Coating

- Cell culture ware 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-XF** or Cardiomyocyte Maintenance **Basal Medium** at or below **4°C** and **Supplement** at **-80°C**.
- Thaw the **Supplement** overnight at 4°C in the dark.
- Add all the **Supplement** to the Cardiomyocyte Maintenance **Basal Medium-XF** or **Basal Medium**.
- For long-term storage, aliquot freshly-prepared **Cardiomyocyte Maintenance Medium-XF** or **Cardiomyocyte Maintenance Medium** and store at **-80°C**. The **Cardiomyocyte Maintenance Medium-XF** or **Cardiomyocyte Maintenance Medium** is then stable for 6 months from the first expiry date of medium/supplement (please refer to the Certificate of Analysis).

Plating Medium

- When ready to use, thaw an aliquot of **Cardiomyocyte Maintenance Medium-XF** or **Cardiomyocyte Maintenance Medium** overnight in the dark at **4°C**.
- Take an aliquot of **Cardiomyocyte Maintenance Medium-XF** or **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**. **NOTE:** 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	Volume added to 50 ml of Medium
Y-27632 dihydrochloride (ROCK inhibitor)	10 mM	10 µM	50 µl
Fetal bovine serum (FBS)	NA	10%	5 ml

MyoMax Maturation Medium

- Upon receipt store **MyoMax Maturation Basal Medium** at or below 4°C and **MyoMax Maturation Supplements A, B and C** at -20°C.
- Add 15 ml of **Supplement A** to the MyoMax Maturation Basal Medium
- Add 9 ml of **Supplement B** to the MyoMax Maturation Basal Medium
- Add 1 ml of **Supplement C** to the MyoMax Maturation Basal Medium
- Complete **MyoMax Maturation Medium** can be stored at 4°C for up to one week.

Thawing and Plating of axoCells™ Ventricular Cardiomyocytes

- On the day of thawing **axoCells™ Ventricular Cardiomyocytes**, prepare the **Cardiomyocyte Maintenance Medium-XF** or **Cardiomyocyte Maintenance Medium**, or have an aliquot of the complete medium thawed, overnight in the dark at 4°C.
- Prepare **Plating Medium** by supplementing Cardiomyocyte Maintenance Medium-XF or Cardiomyocyte Maintenance Medium with 10% FBS and 10 µM Y-27632.
- 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 of the ice has melted, ~ 2 minutes.
- **Do not** shake the vial whilst thawing.
- Take the vial of cells to a biological cabinet, spray 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 transfer the cells into a 15 mL sterile conical tube containing 8 mL **Plating Medium**. Gently rinse the cryovial with 1 mL of fresh **Plating Medium** and transfer the medium with remaining cells into the 15 mL sterile conical tube

- Centrifuge the cells at 200 x 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 or use an automated cell counter. Count the cells and calculate percentage viability.
- 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, taking care to work quickly so that the plate does not dry out.
- Plate the cells at a density of 180,000 – 220,000 cells/cm².

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 per well in a 6-well plate, 1 ml 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% CO₂.
- Using the table below as your guide, the day after plating (day 1), completely and gently replace the culture medium with fresh, pre-warmed (37°C) **Cardiomyocyte Maintenance Medium-XF** or **Cardiomyocyte Maintenance Medium** (without 10% FBS or Y-27632 2HCl (ROCK inhibitor) to remove dead cells/debris. Incubate the cells for 48 hours (to day 3).

Maturation of axoCells™ Ventricular Cardiomyocytes

- On day 3, perform a full media change with **Cardiomyocyte Maintenance Medium-XF** or **Cardiomyocyte Maintenance Medium**. Incubate the cells for 48 hours (to day 5)
- Prepare complete **MyoMax Maturation Medium**.
- On day 5, perform a quarter media change by removing 25% of the spent medium and replacing with the same volume of fresh, pre-warmed (37°C) **MyoMax Maturation Medium**.
- On day 7, perform a half media change by removing 50% of the spent medium and replacing with the same volume of fresh, pre-warmed (37°C) **MyoMax Maturation Medium**.

- Some cell death is expected during the maturation process. Cardiomyocytes will begin to exhibit a more rod-shaped morphology.
- After 7 days in culture, **Ventricular Cardiomyocytes** will be matured and ready for experimental assays.

	Day 0 Thaw & Plate	Day 1 Recovery feed	Day 3 Feed	Day 5 Feed	Day 7 Feed
Maintenance <i>For standard culture of cardiomyocytes which exhibit typical hiPSC-CM characteristics</i>	Plating Medium <i>Cardiomyocyte Maintenance Medium-XF</i> or <i>Cardiomyocyte Maintenance Medium</i> supplemented with 10% FBS and 10 μ M Y-27632	Cardiomyocyte Maintenance Medium-XF or Cardiomyocyte Maintenance Medium	Cardiomyocyte Maintenance Medium-XF or Cardiomyocyte Maintenance Medium	Cardiomyocyte Maintenance Medium-XF or Cardiomyocyte Maintenance Medium-XF	
Maturation <i>For advanced metabolic maturation of cardiomyocytes and more adult-like characteristics</i>				25% MyoMax Maturation Medium	50% MyoMax Maturation Medium

Troubleshooting

MyoMax Maturation Medium is a strong metabolic maturation medium and rapid transition from glycolysis to fatty acid oxidation is stressful for cells. If high amounts of cell death or loss of activity occurs:

- Attempt a more gradual transition from Cardiomyocyte Maintenance Medium to MyoMax maturation medium.
- Follow the protocol **without** the addition of Supplement C to MyoMax Maturation Medium. Cell survival should improve at the expense of maturity.

Got any questions? Need help with the protocol?

Contact Axol Technical Support

at support@axolbio.com

Or

Call +44 (0) 131 651 9710