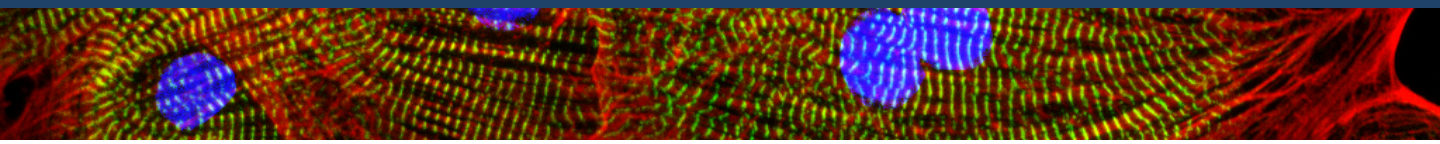
How to...

Culture Human iPSC-derived Atrial and Ventricular Cardiomyocytes on an Alpha MED Microelectrode Array (MEA)



This protocol will take you through the steps of preparing Axol's Cardiomyocyte Maintenance and Plating Medium and setting up the MEA plate along with thawing and seeding Human iPSC-derived Atrial Cardiomyocytes or Ventricular Cardiomyocytes.

Highlighted products used in this application note and where to find them

Product Name	Product Code	Supplier
Human iPSC-derived Atrial Cardiomyocytes	ax2515	Axol Bioscience
Human iPSC-derived Ventricular Cardiomyocytes	ax2505	Axol Bioscience
Fibronectin Coating Solution	ax0049	Axol Bioscience
Cardiomyocyte Maintenance Medium	ax2530	Axol Bioscience
MED64 Presto		Alpha MED Scientific Inc
Cloning ring	11-0162 (RING-05)	Iwaki
CellSpotter24 - Comfort		Alpha MED Scientific Inc
MEA 24-well Plate-comfort	MED-Q2430L	Alpha MED Scientific Inc

Prepare the Media

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 -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 at 4°C 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 Plating Medium.
- Before use, pre-warm an aliquot of Plating Medium at 37°C.

	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

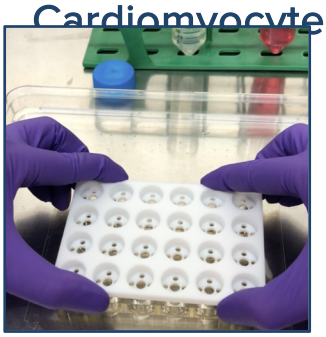
Preparing MEA plates

- Prepare a 1x working stock of fibronectin by diluting the 1mg/mL stock to a final concentration of 40 µg/mL in sterile water e.g. 400 µL in 10 mL.
- The day before thawing the cells, coat the surface of an MEA 24-well plate-comfort, with 500 µL per well of fibronectin (1x working solution - at a concentration of 200 µL per cm^2).
- Gently rock the MEA plate back and forth to evenly distribute the fibronectin.
- Incubate the MEA plate overnight at 37°C, 5% CO_2.
- Remove the diluted Fibronectin from the MEA plate before plating cells.

Thawing Human iPSC-derived Atrial Cardiomyocytes or Ventricular Cardiomyocytes

- Transfer cells from liquid nitrogen into a 37°C water bath, quickly thaw the vial of iPSC-derived cardiomyocytes. 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.
- Transfer the vial into biosafety cabinet spraying thoroughly with 70% ethanol.
- Transfer the cells from the vial and dispense drop-wise into 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.
- Centrifuge the cells at 300 *x g* for 5 minutes at room temperature
- Carefully aspirate and discard the supernatant.
- Gently resuspend the cell pellet in 1 mL of Plating Medium.
- Perform a cell count, suggested seeding density 75,000 cells per cloning ring.

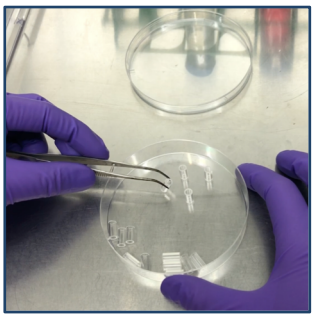
Seeding Human iPSC-derived Atrial & Ventricular Cardiomyocytes

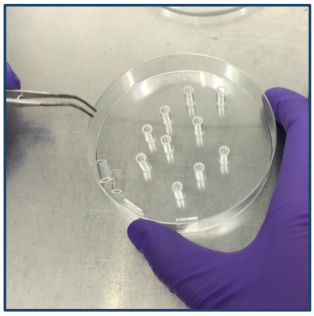


Place the CellSpotter-Comfort (AlphaMED) into the MEA 24-well plate-comfort

Do not remove the coating solution until ready to add the iPSC-derived Atrial or Ventricular Cardiomyocytes.

In a separate sterile dish stand the cloning rings upright



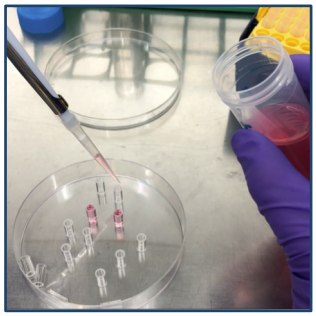


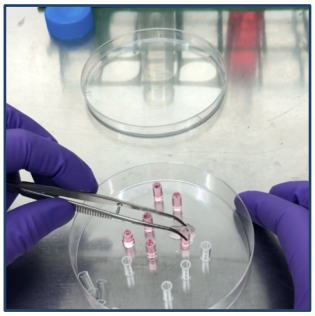
Test the stability of the cloning rings by tapping the dish. If they fall over turn them upside down to ensure you only select the stable cloning rings.

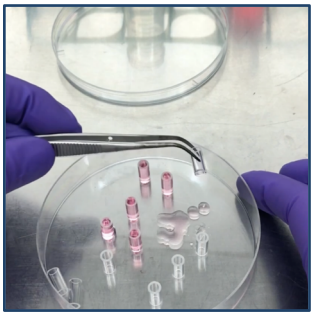
This test ensures that the cloning rings are flat, if the bottom of cloning ring is not flat, the cells can escape during seeding.

Wet 24 cloning rings by adding 100µL of Plating Medium into them.

This will prevent the cell suspension from sticking within the cloning ring.





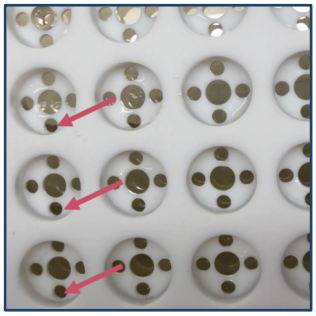


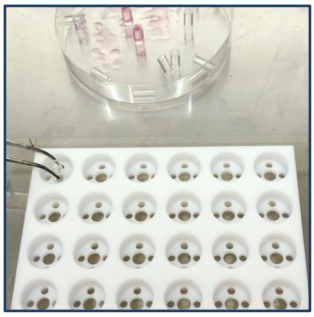
Gently tap the cloning rings on a sterile surface to remove the media.

Take care to make sure cloning rings remain sterile.

With the CellSpotter still in place, remove the Fibronectin coating solution from the MEA plates using the outer holes of the CellSpotter.

Never place pipette in the centre hole, this is where the electrodes are situated.

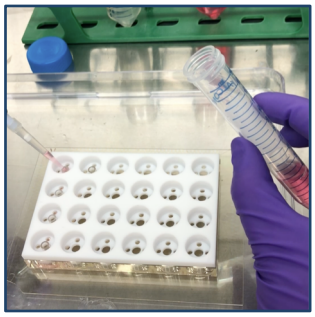


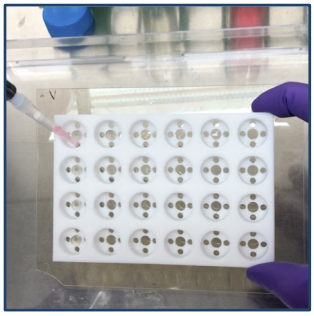


Add one cloning ring per well into the central hole of the CellSpotter

Dispense the calculated volume of cells into the cloning ring and add additional Plating Medium to make the volume in the ring up to 100 µL.

Work quickly as coating solution starts to dry as soon as it is removed.



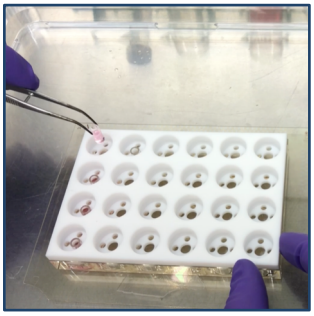


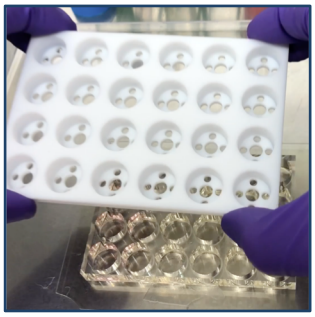
Add 200 µL/cm^2 of Plating Medium into the well surrounding the cloning ring, repeat for each well.

Incubate the MEA plate overnight at 37ºC, 5% CO_2.

The next day gently remove the cloning rings and cell spotter.

There will be medium left in the cloning rings but the cells will have settled and adhered to the MEA plate during the incubation.





Replace the culture medium with fresh, pre-warmed, 37ºC, Cardiomyocyte Maintenance Medium (without 10% FBS or Y-27632 2HCl (ROCK inhibitor)).

Culturing Human iPSC-derived Cardiomyocytes

- Every 2 days conduct a full medium change with fresh, pre-warmed, 37ºC, Cardiomyocyte Maintenance Medium.
- It is important to note that the cells should be incubated for at least 2 hours after changing the medium before taking any electrical recordings. *Changing the medium can cause the beat rate to slow temporarily.*

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
International phone **+44-1223-751-051**
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