

***MEA Application Note:***  
**Primary Culture Cardiac Myocytes**  
**from Chicken Embryo**

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# 1 Material

## 1.1 Biological Materials

- 4 or more chicken eggs (*Gallus gallus*) day E13

## 1.2 Technical Equipment

- Sterile workbench
- Egg incubator
- Incubator at 37 °C, 5 % CO<sub>2</sub>
- Ice
- Centrifuge (for 50 mL tubes)
- 8–10 sterile MEAs (microelectrode arrays)
- Sharp forceps
- Large forceps
- Curved forceps
- Small scissor
- 10 mL pipette
- Petri dishes
- 50 mL Falcon tubes
- 15 mL Falcon tubes
- 100 µm nylon mesh cell strainer



## 1.3 Chemicals

- 3%/10% FCS medium: DMEM/Nutrient mixture F-12 HAM (Sigma D8062) + 3%/10 % FCS + Penicillin Streptomycin
- 0.05 % Trypsin / PBS– (trypsin: Sigma T 7409; PBS–)
- Cellulose Nitrate
- 100 %Methanol
- 70 % Ethanol
- PBS– (without Ca/Mg)

## 2 Methods

### 2.1 Preparations

1. Place 4 or more fertilized eggs into an egg incubator for 13 days.
2. Treat the desired number of MEAs with a plasma cleaner for 2 min.
3. Autoclave the MEAs or sterilize by UV radiation (see MEA User Manual).
4. Coat the recording area of the MEAs with cellulose nitrate solution (see MEA User Manual). This step can be done immediately before the preparation or even during the later incubation steps.
5. Prepare three petri dishes with 20 mL, 10 mL, and 2 mL 3% FCS medium.

### 2.2 Harvesting Cardiac Tissue

1. Remove the embryonic chickens from the eggs and decapitate the chickens in a petri dish filled with 20 mL 3% FCS medium.
2. Open the thoracic regions and isolate the hearts.
3. Gather the hearts in a petri dish filled with 10 mL 3% FCS medium.
4. Remove the atria and vascular tissue, wash the blood away, and transfer the hearts into a dish with fresh medium (2 mL).
5. Chop the hearts with a scissor into small pieces (as small as possible).
6. Collect cardiac fragments with a 10 mL pipette and transfer them into a 50 mL tube. Let the heart pieces sink down inside the pipette so that only a small volume of liquid (about 1 mL) is taken over to the tube.





### 2.3 Digestion with Trypsin

Incubate the heart fragments in 0.05% trypsin/PBS– at 37 °C, as described in the following.

1. 10 min, 10 mL trypsin, discard supernatant.
2. 8 min, fresh 3.5 mL trypsin, collect the supernatant in 10% FCS medium (on ice).
3. 8 min, fresh 3.5 mL trypsin, collect the supernatant in 10% FCS medium (on ice).
4. 8 min, fresh 3.5 mL trypsin, collect the supernatant in 10% FCS medium (on ice).
5. 8 min, fresh 3.5 mL trypsin, collect the supernatant in 10% FCS medium (on ice).
6. Pass the collected cell suspension through a 100 µm nylon mesh cell strainer into a new 50 mL tube.
7. Centrifuge the cell suspension for 10 min at 1500 rpm.
8. Discard the supernatant and resuspend the pellet in 100 µl 3% FCS medium.

### 2.4 Plating Cells onto the MEA

Cells are plated in a high density onto the electrode array. Therefore, the cell culture begins to beat spontaneously after 1–2 days in culture. The cell suspension should be sufficient for about 10 microelectrode arrays (MEA).

1. Plate 10–20 µl cell suspension (equals 150 000–300 000 cells) directly onto the MEA, but avoid touching the electrodes with the pipette. (Electrodes may be damaged.)
2. After a few minutes (the cell suspension should not dry out), carefully fill the MEA with 1 mL 3% FCS medium.
3. Incubate the MEAs in an incubator at 37 °C and replace the medium daily. For incubation over the weekend, you should use as much medium as possible and place the MEA into a water-filled dish to prevent osmotic effects due to evaporation.
4. Recording can be performed from two days after the preparation on until ten days after the preparation. Please note that cell properties change slightly over culture time. Thus, compare only cultures of approximately the same age.

## 3 Suggested MEA System

### 3.1 System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, various system configurations are recommended for the recording from cardiac myocytes.

- **MEA60-Inv-System-E: 60-channel MEA recording system for inverted microscopes.** The **temperature controller** TC01/ TC02 regulates the temperature of the MEA and of the perfusion fluid via **the perfusion cannula** PH01. **1 MEA amplifier** allows recording up to 60 channels from one MEA. This is the standard configuration for low-throughput academic research.
- **MEA60-Inv2-System-E:** This system operates **2 MEA amplifiers** with a 64-channel data acquisition card. It allows recording 30 channels per MEA, on two MEAs simultaneously.
- **MEA120-Inv2-System-E / MEA60-Inv4-System-E:** These systems are based on a **128 channel** data acquisition card and allow the simultaneous operation of **two / four amplifiers**. These systems provide highest data quality and a throughput suitable for both basic research and industrial applications.
- **MEA96MT System / QT-Screen:** These systems record from **96 well plates** with a **single electrode per well**. Whereas the MEA96MT system is based on MC\_Rack and for a manual operation, the QT-Screen provides fully automated liquid handling and analysis. This is a high-end system for high content drug screening.

### 3.2 Microelectrode Arrays

Cardiac myocytes tend to form gap junction coupled cultures that are triggered by a single pacemaker. The question, which MEA type should be used, depends mostly on the size of the area of interest. We recommend an electrode diameter of 30  $\mu\text{m}$  for best signal quality.

Recommended MEAs include:

- **MEA200/30 i. r.** (with substrate-integrated reference electrode) for establishing the cell culture and recording routine
- **ThinMEA200/30 i. r.** for high-resolution imaging and combination with intracellular calcium measurements
- **EcoMEA** for routine recordings with a medium throughput
- **96 well QT plates** for high throughput applications (for use with the MEA96MT System or QT-Screen system)

### 3.3 Amplifier Specifications

Though custom amplifiers with gain and bandwidth specified by the user are available, Multi Channel Systems recommend the following settings for this application.

- Lower cut-off frequency: **1 Hz**  
If you select an even lower value for the lower end of the bandwidth, slow signal drifts will disturb the recordings. Lower limits of up to 10 Hz are recommended if you are not interested in Ca currents, for example for safety screening or mapping of excitation spreading.
- Upper cut-off frequency: **3 kHz**  
Sufficient even for the rapid depolarization waveforms
- Gain: **1200**

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