

MEA Application Note:
Cortical and Hippocampal
Cryopreserved Neurons



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Multi Channel Systems

MCS GmbH

Aspenhaustraße 21

72770 Reutlingen

Germany

Fon +49-71 21-90 92 5 - 0

Fax +49-71 21-90 92 5 -11

info@multichannelsystems.com

www.multichannelsystems.com

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A major part of this information is based on the instructions provided by members of the Neurochip consortium in Düsseldorf, Prof. Mario Siebler and Wiebke Fleischer; and the staff of QBM Cell Science, especially Dr. Babben Tinner-Staines.

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1 Introduction

1.1 About this Application Note

The intention of the MEA Application Notes is to show users how to set up real experiments with the MEA System on the basis of typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

This application note includes a complete protocol for the cultivation of *ready-to-use* cryopreserved primary neurons, suggestions for long term cultures, suggestions for MEA System configurations, example data, and references.

1.2 Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us, especially the following persons.

Wiebke Fleischer

Babben Tinner-Staines

2 Material

2.1 Biological Materials

- Ready to use cryopreserved primary neurons (from hippocampus or cortex) from QBM Cell Science

2.2 Technical Equipment

- MEA System (with amplifier and data acquisition, see [Suggested MEA System](#))
- Stimulus generator
- MEAs (microelectrode arrays)
- Sterile workbench
- Incubator set to 35 °C, 65 % relative humidity, 9 % O₂, 5 % CO₂
- Water bath at 37 °C
- Liquid nitrogen freezing tank
- Stereo microscope
- Inverted microscope
- Micropipettes and pipette tips (1000 µL)
- 15 mL BD Falcon tubes
- Teflon membranes (ALA Scientific Instruments)

2.3 Chemicals

- 70 % alcohol for disinfection
- Polyethylenimine (PEI)
- Laminin
- Glutamine
- Neurobasal Medium with B27 supplement (Gibco/Invitrogen)
- [Optional: penicillin/streptomycin]

2.4 Media

- Neurobasal medium
- B-27 supplement
- 0.5 mM Glutamine
- [optional: 50 U/ml penicillin/streptomycin]

This medium is recommended by QBM Cell Science, the provider of the cryopreserved neurons.

3 Methods

3.1 MEA Coating

Depending on the type of selected MEA, various coatings may be applied. Standard MEAs should be coated with Polyethylenimine or Laminin. Suggestions for coating methods can be found in the MEA User Manual available in the [Download section](#) of the MCS web site.

3.2 Thawing and Starting the Culture

IMPORTANT: Do not centrifuge or vortex the cells. Keep the time between removing the vial from the liquid nitrogen tank and placing it into the water bath as short as possible.

1. Store the cells in liquid nitrogen until use.
2. Remove a vial with the cells from the liquid nitrogen and place it into a water bath pre-heated at 37 °C to thaw the cells.
3. After 2 ½ minutes, remove the vial with the thawed cells and disinfect the outside of the vial with 70 % ethanol. Place it under a laminar flow hood. Proceed with the next step immediately after thawing.
4. Gently transfer 1 ml of the cells into a 15 ml centrifuge tube and immediately add 37 °C pre-warmed medium drop-wise onto the cells, while rotating the tube by hand. This procedure should take approximately 2 minutes. We recommend addition of 4 mL medium resulting in a final cell density of 800.000 per mL.

IMPORTANT: Do not add the whole volume of the medium to the cells at once. This may result in an osmotic shock. If the same vial is to be used for several different experiments in parallel, mix the cells by pipetting slowly up and down once, then aliquot the cells into the appropriate vessels.

5. Mix the cell suspension by inverting the tube carefully, twice.
6. Transfer the cell suspension into the MEA culture chamber, about 0.6–1.6 mL (depending on the maximum volume of the MEA culture chamber).
7. Incubate the cells in an incubator for 4 hours.
8. Remove the medium from the cells leaving a small liquid layer to ensure that the cells do not dry out and add 1.6 mL fresh, 37 °C pre-warmed medium.
9. Incubate the cells at 37 °C with 95 % relative humidity and 5 % CO₂ until use.

IMPORTANT: Cell death at the beginning of the cultivation should be considered normal. Generally, there are enough viable cells left for cultivation.

3.3 Maintenance

- Change the medium at day 5 after thawing.
- Replace 50 % of the medium with fresh, 37 °C pre-warmed medium every 3 to 4 days. Warm an appropriate amount of medium to 37°C in a sterile tube. Remove 50 % of the medium from the cell culture. Replace with the warmed, fresh medium and return the cells to the incubator.
- Avoid repeated warming and cooling of the medium. Warm only the volume that is needed for a single procedure.
- Compensation for media loss due to evaporation should be taken into consideration. Add additional media whenever necessary.
- After 2–3 weeks of cultivation, the cells are ready for recording.

4 Longterm Culturing

In order to allow long term cultivation and recording, Multi Channel Systems recommends the use of teflon membranes (fluorinated ethylene-propylene, 12.5 microns thick) developed by Potter and DeMarse (2001). The ALA-MEA-MEM membrane is produced in license by ALA Scientific Instruments Inc., and distributed via the world-wide network of MCS distributors.



The sealed MEA culture chamber with transparent semipermeable membrane is suitable for all MEAs with glass ring. A hydrophobic semipermeable membrane from Dupont that is selectively permeable to gases (O_2 , CO_2), but not to fluid, keeps your culture clean and sterile, preventing contaminations by airborne pathogens. It also greatly reduces evaporation and thus prevents a dry-out of the culture.

5 Suggested MEA System

5.1 System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, different system configurations are recommended for the recording from cultured neurons.

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. One **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 a throughput suitable for both basic research and industrial applications.

5.2 Microelectrode Arrays

Available MEAs differ in electrode material, diameter, and spacing. For an overview on available MEA types please see the Multi Channel Systems web site (www.multichannelsystems.com) or contact your local retailer.

The microfold structures formed by titanium nitride (TiN) result in a large surface area that allows the design of small electrodes with a low impedance and an excellent signal to noise ratio. For recording from cultured neurons, a medium spatial resolution with an electrode diameter of 30 μm and a spacing of 200 μm is generally sufficient.

Recommended MEAs include:

- **MEA 200/30 i. r.:** standard 8 x 8 layout, TiN electrodes for recording and stimulation, with substrate-integrated reference electrode
- **ThinMEA 200/30 i. r.** for high-resolution imaging and combination with intracellular calcium measurements. ThinMEAs are only 180 μm "thick" and mounted on a robust ceramic carrier. Tracks and contact pads are made of transparent ITO.

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