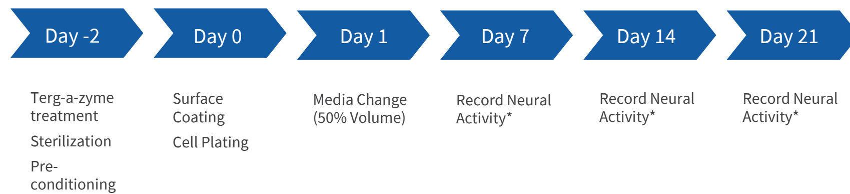
MAXWELL BIOSYSTEMS

MaxTwo Neuronal Cell Plating Protocol

1. Experiment Workflow



* *Other experiments may require specific frequency of media changes and timing of neural activity recording.*

2. Required Materials

Item	Supplier	Catalog Number
MaxTwo Multi-Well Plate (with Lid, autoclave Lid before use)	MaxWell Biosystems AG	MX2-S-6W
MaxTwo Mainframe	MaxWell Biosystems AG	MX2-SYS
Primary Neurons (cortical, hippocampal, etc.)	Transnetyx* In-house generated cells	SKU SDEHP
Human iPSC-derived Neurons	Fujifilm Cellular Dynamics* bit.bio* Elixirgen Scientific* BrainXell* In-house generated cells	R1088 io1001 EXGS-QNMSV BX-0500
Borate Buffer 20X	ThermoFisher Scientific	28341
Cell Culture Media	Multiple Vendors	
Ethanol 70%	Multiple Vendors	
Sterile Deionized Water	Multiple Vendors	
Surface Coating Material	See section 4 and 5	
Sterile Syringe Filter (0.22 µm pore)	Multiple Vendors	
Terg-a-zyme	Sigma-Aldrich	Z273287
Breathe-Easy® Sealing Membrane	Sigma-Aldrich	Z380059
Nunc™ Square BioAssay Dishes (optional)	ThermoFisher Scientific	240835
Low Retention Tip (optional)	Multiple Vendors	
Incubox™ (optional)**	Insphero	

* *Example suppliers and neuronal cell lines, which were tested using MaxOne and MaxTwo.*
If you are interested in establishing a protocol for your cells, contact us at info@mxwbio.com.

** *Contact support@mxwbio.com for order details.*

3. MaxTwo Multi-Well Plate Surface Preparation [A]

Procedure

1. Prepare a 1%-Terg-a-zyme-solution (10 g/L) in deionized water. Always use fresh 1%-Terg-a-zyme solution.
2. Add 2 mL of 1%-Terg-a-zyme-solution into each well of the MaxTwo Multi-Well Plate and incubate at room temperature for **2 hours**.
3. Remove the 1%-Terg-a-zyme-solution and wash each of the MaxTwo Multi-Well Plate wells three times with deionized water. Ensure that the 1%-Terg-a-zyme-solution is completely washed out.

Notes

Change 50% of the media three times a week from Day 1 onwards (or as recommended by the supplier).

Recording session is recommended between 4 to 24 hours after medium change.
It is recommended to fix the duration between medium change and recording session.

Plating co-cultures of neurons and astrocytes is an alternative approach.

A: *These steps are crucial for the hydrophilicity of the chip surface.*

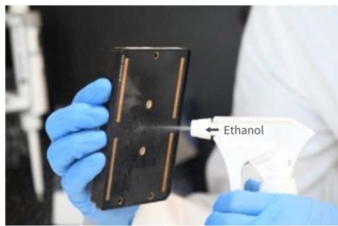


Figure 1. Spraying MaxTwo Multi-Well Plate with 70% Ethanol

Figure 2. Filling the Wells of MaxTwo Multi-Well Plate with 70% Ethanol

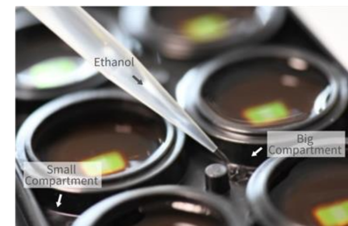


Figure 3. Filling Compartments between Wells with 70% Ethanol

4. MaxTwo Multi-Well Plate Sterilization and Pre-conditioning [A]

Procedure

1. **For sterilization:** Spray the MaxTwo Multi-Well Plate front and back thoroughly with 70% ethanol (see Figure 1).
2. Fill each well and each compartment of the MaxTwo Multi-Well Plate with 70% ethanol (see Figures 2 and 3).
3. Transfer the MaxTwo Multi-Well Plate to the biological safety cabinet.
4. Remove the 70% ethanol from the wells and compartments after **30 mins**.
5. Wash each well of the MaxTwo Multi-Well Plate three times with sterile deionized water.
6. Aspirate the water with a vacuum pump and dry the bottom of the MaxTwo Multi-Well Plate **[B]**.
7. **For pre-conditioning:** Fill each well of the MaxTwo Multi-Well Plate with 1.2 mL of complete cell culture media.
8. Cover the MaxTwo Multi-Well Plate with a Breathe-Easy® sealing membrane, and then place the autoclaved lid on top.
9. Keep the MaxTwo Multi-Well Plate inside the 5% CO_2 incubator at 37°C, relative humidity (RH) >95%, for **2 days**.
10. Before the cell plating, aspirate the complete cell culture medium from the MaxTwo Multi-Well Plate and wash each well once with sterile deionized water. Completely aspirate the water from each well with a vacuum pump. Proceed to surface coating.

***B:** Make sure that the bottom of the MaxTwo Multi-Well Plate is dry!*

5. MaxTwo Multi-Well Plate Surface Coating

Primary Coating Chemical Options

Chemical	Poly(Ethyleneimine) (PEI)	Poly-D-Lysine (PDL) hydrobromide
Supplier	Sigma-Aldrich	Sigma-Aldrich
Catalog Number	P3143	P6407
Working Concentration	0.07%	0.1 mg/ml
Incubation Location	37°C 5% CO_2 incubator	37°C 5% CO_2 incubator
Incubation Time	1 hour	3 hours
Preparation	Dilute stock in sterile borate buffer. See preparation of PEI.	Dilute stock in sterile borate buffer. See preparation of PDL.

Preparation of PEI

1. Prepare 100 mL of 1X borate buffer: dilute 5 mL of 20X borate buffer in 95 mL sterile deionized water.
2. Prepare a 7% PEI stock solution: Weigh 1.4 g of 50% PEI solution directly into a sterile beaker. Prepare the 7% PEI stock solution by adding 8.6 mL of 1X borate buffer into the sterile beaker. Mix for approximately 30 mins using a magnetic stirrer until fully dissolved (can be stored in 0.5 mL aliquots at -20°C for 1 month).
3. Prepare a final 0.07% PEI solution by diluting 0.5 mL of intermediate 7% PEI solution in 49.5 mL of 1X borate buffer fresh before use.
4. For sterilization before use, filter through a 0.22 µm filter unit.

Preparation of PDL

1. Prepare 100 mL of 1X borate buffer: dilute 5 mL of 20X borate buffer in 95 mL sterile deionized water.
2. Prepare a 100 µg/mL suspension: Resuspend 5 mg of PDL in 1x borate buffer. Aliquots can be stored at -20°C.
3. For sterilization before use, filter through a 0.22 µm filter unit.

Procedure for Primary Coating

1. Add 50 µL of the Primary Coating Solution to the center of each well of the MaxTwo Multi-Well Plate, covering the entire electrode array **[C].**
2. Cover the MaxTwo Multi-Well Plate with Breathe-Easy® sealing membrane, and then place the autoclaved lid on top.
3. Incubate MaxTwo Multi-Well Plate for **1 hour** in a 5% CO_2 incubator at 37°C, RH >95%.
4. Aspirate Primary Coating Solution completely.
5. Wash each well of the MaxTwo Multi-Well Plate three times with 1 mL of sterile deionized water.
6. Aspirate the sterile deionized water with a vacuum pump. Let the MaxTwo Multi-Well Plate dry inside the biological safety cabinet for **1 hour**.

***C:** Gently pipette the coating solution onto the center of each electrode array. Avoid touching the surface with the tip of the pipette!*

Secondary Coating Chemical Options

Chemical	Laminin	Geltrex
Company	Sigma-Aldrich	ThermoFisher Scientific
Catalog Number	L2020	A1413202
Working Concentration	0.02 mg/mL	1:100
Incubation Location	37°C 5% CO_2 incubator	37°C 5% CO_2 incubator
Incubation Time	1 hour	1 hour
Preparation	Dilute stock in cell culture medium.	Dilute stock in cell culture medium. See preparation for Geltrex.

Preparation of Geltrex

1. Thaw Geltrex stock on ice in a 4°C fridge overnight. Prepare aliquots. Thaw aliquots 30 mins on ice.
2. While using Geltrex keep solution on ice, otherwise it solidifies.
3. Dilute Geltrex 1:100 in chilled medium.

Procedure for Secondary Coating [D]

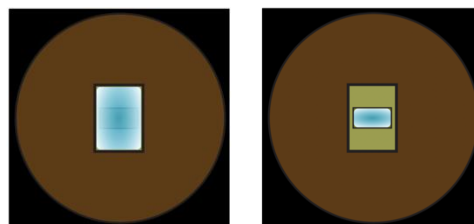
1. For Cell Plating VERSION A, add **50** µL of Secondary Coating Solution to the center of each well of the MaxTwo Multi-Well Plate (see Table below), for Cell Plating VERSION B, add **5** µL of Secondary Coating Solution to the center of each well of the MaxTwo Multi-Well Plate (see Table below).
2. Cover the MaxTwo Multi-Well Plate with a Breathe-Easy® sealing membrane, and then place the autoclaved lid on top.
3. Incubate MaxTwo Multi-Well Plate for **1 hour** in a 5% CO_2 incubator at 37°C, RH >95%.
4. Aspirate Secondary Coating Solution (do not wash after aspirating the Secondary Coating Solution) and immediately proceed to cell plating.

__D:__ Instead of a secondary coating, an embedding can be performed. Here the cells are directly added to the surface within the secondary coating solution.

6. Cell Plating

Procedure for Cell Plating

Choose between two different plating options:

	VERSION A Whole-Area Plating	VERSION B Dot Plating
Area	~50 mm²	~10 mm²
Cell drop volume	50 µL	5 µL
Primary neurons		
Cell count per drop	100’000-150’000	20’000-30’000
Cell density (cells/mm²)	~2’000-3’000	~2’000-3’000
Cell concentration (cells/µL)	2’000-3’000	4’000-6’000
Human iPSC-derived neurons		
Cell count per drop	500’000-750’000	100’000-150’000
Cell density (cells/mm²)	~10’000-15’000	~10’000-15’000
Cell concentration (cells/µL)	10’000-15’000	20’000-30’000
Picture of plating area		

Practice the Dot Plating before the first plating!

1. Add cell solution to each well of the MaxTwo Multi-Well Plate at the center of the electrode array according to your plating option (VERSION A: Whole Area, VERSION B: Dot) (see Figure 4) **[E].**

__E:__ Make sure to regularly mix the cell suspension when plating several MaxTwo Multi-Well Plates to prevent the cells from settling at the bottom of the tube.

Try to position the cell suspension as close to the center of the electrode array as possible. Low Retention Tips are recommended for this step.

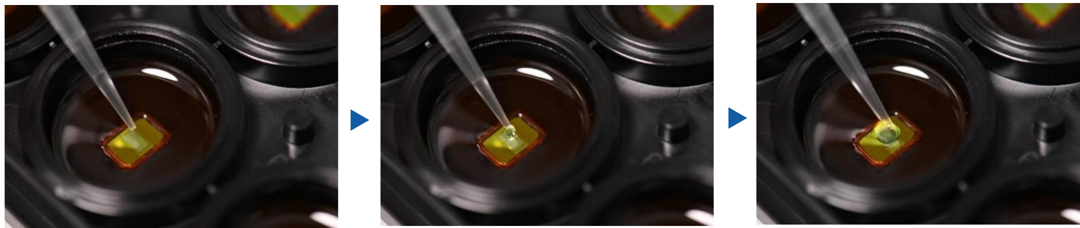


Figure 4. Dot Plating, VERSION B

2. Cover the MaxTwo Multi-Well Plate with a Breathe-Easy® sealing membrane, and then place the autoclaved lid on top **[F].**
3. Incubate MaxTwo Multi-Well Plate in a 5% CO_2 incubator at 37°C, RH >95%, for **1 hour**.

4. After incubation, carefully fill each well of the MaxTwo Multi-Well Plate with 1.2 mL of complete culture medium. Pipette liquid on the side of the well.

***F:** During cell culture maintenance in the incubator, keep the MaxTwo Multi-Well Plate covered with Breathe-Easy® sealing membrane to prevent evaporation. Alternatively, keep the MaxTwo Multi-Well Plate inside Nunc™ Square BioAssay Dishes or in the IncuBox™ inside the incubator to prevent evaporation.*

7. Maintenance of Cell Cultures and Recording of Neural Activity

1. Change 50% of the complete cell culture media on day 1 after plating (or as recommended by cell supplier).
2. Maintain the cell cultures in a 5% CO_2 incubator at 37°C, RH >95%. Replace 50% of the media three times a week (or as recommended by cell supplier).
3. It is recommended to record neural activity 4 to 24 hours after medium change.
4. A fixed time duration between media change and recording is recommended.
5. Before inserting the MaxTwo Multi-Well Plate into the MaxTwo Mainframe, turn on the device and the gas supply 30 mins prior.
6. After plugging the MaxTwo Multi-Well Plate into the MaxTwo Mainframe, it is recommended to wait 10 mins before starting your recording session.

Conditions of Use

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Revision History

Version 3.2: May 2023

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