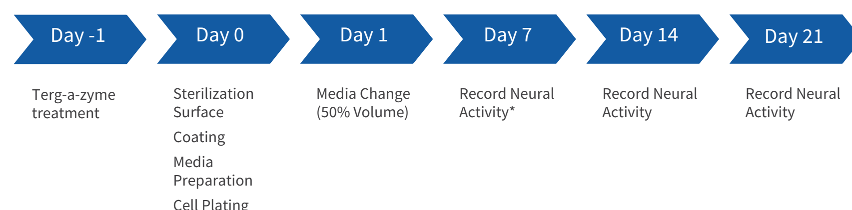
MaxTwo Neuronal Cell Plating Protocol

1. Experiment Workflow



** These are recommended, however other experiments may require specific frequency of media changes and timing of neural activity recording.*

Notes

Change 50% medium twice a week from Day 1.*

2. Required Materials

Item	Supplier	Catalog Number
MaxTwo Multi-Well Plate	MaxWell Biosystems AG	MX2-S-6W
Frozen Primary Rat Neurons	Brain Bits*	FSDECX1M
Human iPSC-derived Neurons	BrainXell* Elixirgen* FCDI*	BX-0500 EXGS-QNMSV R1088
Cell Culture Medium	Multiple Vendors	
Ethanol 70%	Multiple Vendors	
Sterile Deionized Water	Multiple Vendors	
Surface Coating Materials (see section 5)	Multiple Vendors	
Terg-a-zyme	Sigma-Aldrich	Z273287

** These are example sources of neuronal cells that we have tested in MaxOne and MaxTwo. If you are interested to establish a protocol for your cells, contact us at info@mxwbio.com.*

3. MaxTwo Multi-Well Plate Sterilization

Procedure

1. Prepare Terg-a-zyme-solution in deionized water (1% solution: 10 g per liter).
2. Fill each well of the MaxTwo Multi-Well Plate with 1 mL of 1% Terg-a-zyme-solution and leave over night at room temperature (R.T).
3. Wash wells three times with deionized water.
4. Remove the remaining sterile deionized water and spray the MaxTwo Multi-Well Plate and MaxTwo lid thoroughly with 70% ethanol (see Figure 1).
5. Fill each well and each compartment of the MaxTwo Multi-Well Plate with 70% ethanol (see Figure 2).
6. Transfer the MaxTwo Multi-Well Plate to the biological safety cabinet.
7. Remove the 70% ethanol from the wells and compartments after 30 minutes.
8. Rinse each well and compartment of the MaxTwo Multi-Well Plate 3 times with sterile deionized water.
9. Aspirate the sterile water with a vacuum pump. **[A]**
10. Fill the small compartments between the wells with 0.5 mL and the big compartments with 1 mL sterile deionized water (see Figure 3).
11. Cover the MaxTwo Multi-Well Plate with its Lid.
12. Proceed to surface coating.

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

Figure 1: Sterilize the MaxTwo Multi-Well Plate

Figure 2: Fill the wells of MaxTwo Multi-Well Plate with 70% ethanol

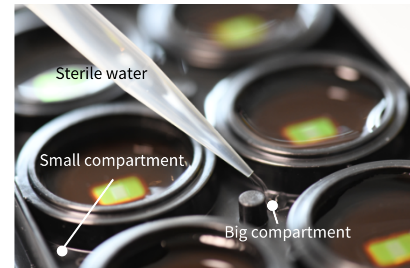


Figure 3: Fill compartments between wells with sterile deionized water

4. MaxTwo Multi-Well Plate Surface Coating

Notes

Primary Coating Chemical Options

Chemical	Poly (Ethyleneimine) (PEI)	Poly-L-Ornithine (PLO)	Poly-D-Lysine (PDL)
Company	Sigma/Merck	Sigma/Merck	ThermoFisher Scientific
Catalog Number	P3143	P4957	A3890401
Working Concentration	0.07%	0.005%	0.1 mg/mL
Incubation Location	37°C 5% CO_2 incubator	37°C 5% CO_2 incubator	37°C 5% CO_2 incubator
Incubation Time	1 hour	2 hours	1 hour
Preparation	Dilute stock in sterile borate buffer See preparation notes PEI	Dilute stock in sterile deionized water See preparation notes PLO	Ready to use

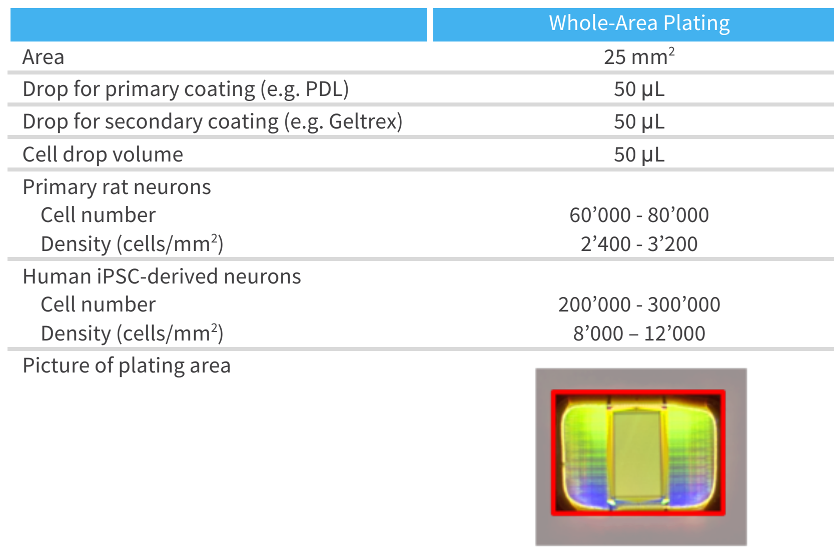
Preparation for PEI

1. Prepare 100 mL of 1X borate buffer: dilute 5 ml of 20X borate buffer (ThermoFisher Scientific 28341) in 95 mL in sterile water.
2. Prepare an intermediate 7% PEI solution: pour 1 mL of 50% PEI solution into a 15 mL centrifuge tube and allow it to settle. Add 6 mL of 1X borate buffer to obtain an intermediate ~7% PEI solution (can be stored in 1 mL aliquots at -20°C for 1 month).
3. Prepare a final ~0.07% PEI solution by diluting 1 mL of intermediate 7% PEI solution in 99 mL of 1X borate buffer.
4. For sterilization, filter through a 0.22 µm filter unit.

Preparation for PLO

1. Mix 1 mL 0.01% PLO stock solution with 1 mL sterile water.
2. For sterilization, filter through a 0.22 µm filter unit.

Surface Coating Area Options



	Whole-Area Plating
Area	25 mm^2
Drop for primary coating (e.g. PDL)	50 µL
Drop for secondary coating (e.g. Geltrex)	50 µL
Cell drop volume	50 µL
Primary rat neurons Cell number Density (cells/mm^2)	 60’000 - 80’000 2’400 - 3’200
Human iPSC-derived neurons Cell number Density (cells/mm^2)	 200’000 - 300’000 8’000 – 12’000
Picture of plating area	

Procedure (example PDL)

1. Add PDL solution to each well of the MaxTwo Multi-Well Plate covering the entire electrode array (see Figure 4). **[B]**
2. Cover the MaxTwo Multi-Well Plate with its Lid.
3. Incubate the MaxTwo Multi-Well Plate with PDL in a 5% CO_2 incubator at 37°C. **[C]**
4. Aspire PDL.
5. Rinse each well of the MaxTwo Multi-Well Plate 3 times with 1 mL sterile deionized water.
6. Aspirate the sterile water with a vacuum pump.

Notes

B: *Gently pipette the coating solution onto the center of each MEA. Avoid touching the MEA surface with the tip of the pipette!*

C: *Adapt incubation time according to primary coating chemical.*

5. Cell Plating

Secondary Coating Chemical Options

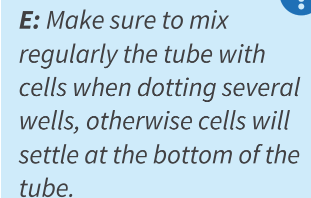
Chemical	Laminin	Matrigel	Geltrex
Company	Sigma/Merck	Corning	ThermoFisher Scientific
Catalog Number	L2020	354230	A1569601
Working Concentration	0.02 mg/mL	0.4 mg/mL	1x
Incubation Location	37°C 5% CO_2 incubator	37°C 5% CO_2 incubator	37°C 5% CO_2 incubator
Incubation Time	30 minutes	2 hours	1 hour
Preparation	Dilute stock in cell culture media	Dilute stock in cell culture media	Ready to use

Procedure (example Geltrex)

1. Add Geltrex solution to the center of each well of the MaxTwo Multi-Well Plate (see Figure 4).
2. Cover the MaxTwo Multi-Well Plate with its Lid.
3. Incubate the MaxTwo Multi-Well Plate with Geltrex in a 5% CO_2 incubator at 37°C. **[D]**
4. Aspirate Geltrex.
5. Add cell solution to each well of the MaxTwo Multi-Well Plate on the area previously covered by Geltrex. **[E]**
6. Cover the MaxTwo Multi-Well Plate with its Lid.
7. Incubate MaxTwo Multi-Well Plate with neurons in a 5% CO_2 incubator at 37°C for 1 hour. **[F]**
8. After incubation, fill up each MaxTwo Multi-Well Plate carefully with 1.2 mL of medium.
9. Change 50% of the medium on day 1 after plating.
10. Maintain the cell cultures inside a 5% CO_2 incubator at 37°C to control environmental conditions. Replace 50% of the medium twice a week.

Notes

***D:** Adapt incubation time according to secondary coating chemical.*

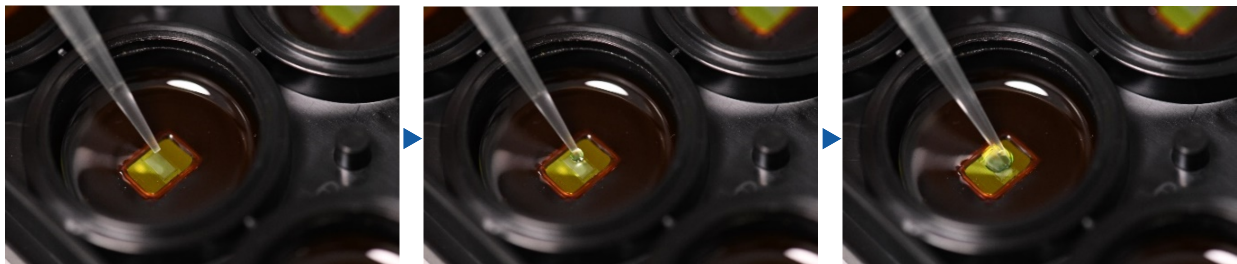


***E:** Make sure to mix regularly the tube with cells when dotting several wells, otherwise cells will settle at the bottom of the tube.*

As much as possible, keep the cell droplet at the center of the electrode array.

***F:** Covering the MaxTwo Multi-Well Plate is crucial to avoid medium evaporation while cells settle down on the surface.*

Figure 4: Placing coating solution



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Revision History
Version 1: November 2020

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