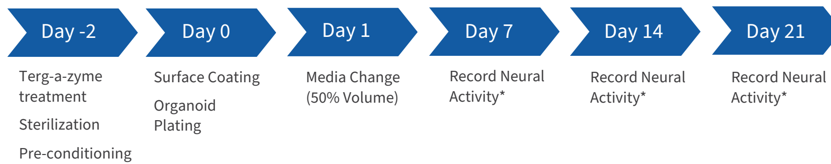
maxwell BIOSYSTEMS

Brain Organoid Plating Protocol

1. Experiment Workflow



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

Notes

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

2. Required Materials

Item	Supplier	Catalog Number
MaxOne Chip - PSM	MaxWell Biosystems AG	MX1-S/U-CHP
MaxOne Lids (autoclave before use)	MaxWell Biosystems AG	MX1-LID-PSM
Borate Buffer 20X	ThermoFisher Scientific	28341
Culture Media	Multiple Vendors	
Sterile Petri Dishes (⌀ 90 mm)	Multiple Vendors	
Sterile Petri Dishes (⌀ 30 mm)	Multiple Vendors	
Ethanol 70%	Multiple Vendors	
Sterile Deionized Water	Multiple Vendors	
Coating Material	See section 4 and 5	
Sterile Glass Beaker	Multiple Vendors	
Terg-a-zyme	Sigma-Aldrich	Z273287
Sterile Filter (0.22 µm pore)	Multiple Vendors	
Fine, Thin Sterile Paint Brushes (size 0)	Multiple Vendors	
Absorption Spears, SUGI	AgnThos	18105-01
ART Wide Bore Filtered Pipette Tips (Optional)	ThermoFisher Scientific	2079G or 2069G

If you are interested in establishing a protocol for your organoids, contact us at info@mxwbio.com.

3. MaxOne Chip Surface Preparation [A]

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

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

4. MaxOne Chip Surface Sterilization and Pre-conditioning [A]

1. **For sterilization**: Transfer the MaxOne Chips into a beaker filled with 70% ethanol (see Figure 1) **[B].**
2. Transfer the beaker into the biological safety cabinet.
3. Take out the MaxOne Chips from the beaker after **30 mins**.
4. Wash the MaxOne Chips thoroughly three times with sterile deionized water.

***B:** Every MaxOne Chip has to be completely soaked in 70% ethanol!*

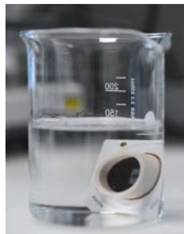


Figure 1. MaxOne Chip sterilization

Figure 2. MaxOne Chip gold contact pads

Figure 3. MaxOne Chip with lid in a humidity chamber

5. Aspirate the water with a vacuum pump.
6. Once the MaxOne Chips are dry (including the bottom of the chips, see Figure 2) **[C]**, place each MaxOne Chip inside a ⌀ 90 mm Petri dish.
7. Make humidity chambers by placing ⌀ 30 mm Petri dishes inside ⌀ 90 mm Petri dishes, next to the MaxOne Chip (see Figure 3).
8. Fill each ⌀ 30 mm Petri dish with 1 mL of sterile deionized water.
9. **For pre-conditioning:** Fill each MaxOne Chip with 0.6 mL complete culture media.
10. Cover the MaxOne Chips with autoclaved MaxOne Lids. Ensure that the media in the well does not touch the lid. Cover the ⌀ 90 mm Petri dish with its lid to finish creating the humidity chamber.
11. Keep the MaxOne Chips in humidity chambers inside the 5% CO_2 incubator at 37°C, relative humidity (RH) > 95%, for **2 days**.
12. Before the plating, aspirate the culture media from the MaxOne Chips and wash each well once with sterile deionized water. Completely aspirate the water from the MaxOne Chips with a vacuum pump. Proceed to surface coating.

C: *Check that the gold contact pads on the bottom of the MaxOne Chips are dry when placing them inside the ⌀90 mm Petri dish (see Figure 2).*

5. MaxOne Chip Surface Coating

Recommended Primary Coating Chemical Option

Chemical	Poly (Ethyleneimine) (PEI)
Supplier	Sigma-Aldrich
Catalog Number	P3143
Working Concentration	0.07%
Incubation Location	37°C 5% CO_2 incubator
Preparation	Dilute stock in sterile borate buffer. See preparation of PEI.

Preparation of PEI

1. Prepare 120 mL of 1X borate buffer: dilute 6 mL of 20X borate buffer in 114 mL sterile deionized water.
2. Prepare an intermediate 7% PEI solution: pour 1 mL of 50% PEI solution into a 15 mL conical 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 up to 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 before use.

Procedure for Primary Coating

1. Add 50 µL of the Primary Coating Solution to the center of each MaxOne Chip, covering the entire electrode array **[D].**
2. Cover each MaxOne Chip with an autoclaved MaxOne Lid.
3. Incubate MaxOne Chips for **1 hour** in a 5% CO_2 incubator at 37°C, RH > 95%.
4. Aspirate the Primary Coating Solution completely.
5. Wash each MaxOne Chip three times with 1 mL of sterile deionized water.
6. Aspirate the sterile deionized water with a vacuum pump. Let the MaxOne Chips dry inside the biological safety cabinet for **60 mins**.

D: *Gently pipette the coating solution onto the center of MaxOne Chip. Avoid touching the MaxOne Chip surface with the tip of the pipette!*

Recommended Secondary Coating Chemical Option

Chemical	Laminin
Company	Sigma-Aldrich
Catalog Number	L2020
Working Concentration	0.04 mg/mL
Incubation Location	37°C 5% CO_2 incubator
Preparation	Dilute stock in culture media.

Procedure for Secondary Coating

1. Add 50 µL of Secondary Coating Solution to the center of each MaxOne Chip, covering the entire electrode array.
2. Cover each MaxOne Chip with an autoclaved MaxOne Lid.
3. Incubate MaxOne Chips for **1 hour** in a 5% CO_2 incubator at 37°C, RH > 95%.
4. Aspirate the Secondary Coating Solution completely (do not wash after aspirating the Secondary Coating Solution) and immediately proceed to organoid plating.

6. Organoid Plating

1. Collect a single or multiple organoid(s) with a pipette and place it on the electrode array in a small droplet **[E]**.
2. If needed, use a thin sterile brush to gently move the organoid(s) into the correct position over the electrode array (see Figure 4). Do not touch the organoid(s) directly with the brush. Instead, use the brush to gently push the liquid around the organoid(s), thereby moving it in the desired direction.

***E:** A Wide Bore Pipette Tip can also be used to transfer organoid(s). Alternatively, the end of a pipette tip can be cut with sterile scissors.*

Figure 4. Position the organoid(s) onto the electrode array

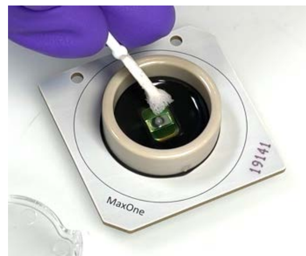


Figure 5. Use a SUGI absorption spear to remove excess liquid

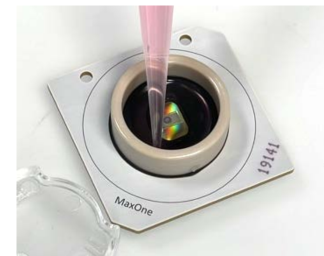


Figure 6. Gently fill the MaxOne Chip with culture media

3. Use a SUGI Absorption Spear to remove excess liquid around the organoid(s) until the surface is <u>completely dry</u>, allowing the organoid(s) to properly adhere to the electrode array (see Figure 5). Steps 2-3 should not exceed ~1 min **[F]**.
4. Leave the organoid(s) for **~1-2 mins** (max. 5 mins) to allow for sufficient attachment. Monitor closely to prevent the organoid(s) from drying out **[G]**.
5. Gently add 50 µL of complete culture media onto the organoid(s), check if the organoid(s) detach. If so, repeat Steps 2-5.
6. Cover each MaxOne Chip with an autoclaved MaxOne Lid. Place the MaxOne Chip inside the humidity chamber and incubate for **2 hours** in a 5% CO_2 incubator at 37°C, RH > 95% **[H]**.
7. After incubation, gently fill the well up to a total volume of 0.6 mL with complete culture media. Allow the media to flow slowly from the pipette tip to prevent detachment (see Figure 6). If the organoid(s) detach, repeat the procedure from Step 2 onwards.
8. Cover each MaxOne Chip with an autoclaved MaxOne Lid and place it in a 5% CO_2 incubator at 37°C, RH > 95%.

***F:** It is important <u>NOT</u> to touch the organoid(s) with the SUGI absorption spear.*

***G:** Optionally, cover the MaxOne Chip with its lid to avoid drying out the organoid(s) during this step. This is important in biological safety cabinets with strong laminar flow.*

***H:** Covering the MaxOne Chip with its lid and placing it inside a humidity chamber is crucial to avoid media evaporation while the organoid(s) settle and adhere to the surface.*

7. Maintenance of Organoid Cultures and Recording of Neural Activity

1. Change 50% of the complete culture media on day 1 after plating (or as recommended by supplier).
2. Maintain the organoid culture in a 5% CO_2 incubator at 37°C, RH > 95%. Replace 50% of the media at least twice a week (or as recommended by supplier).
3. It is recommended to record neural activity a minimum of 24 hours after media change.
4. A fixed time duration between media change and recording is highly recommended.
5. Start the recording session 10 mins after plugging MaxOne Chip into the MaxOne Recording Unit.

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Revision History
Version 2.0: March 2023
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