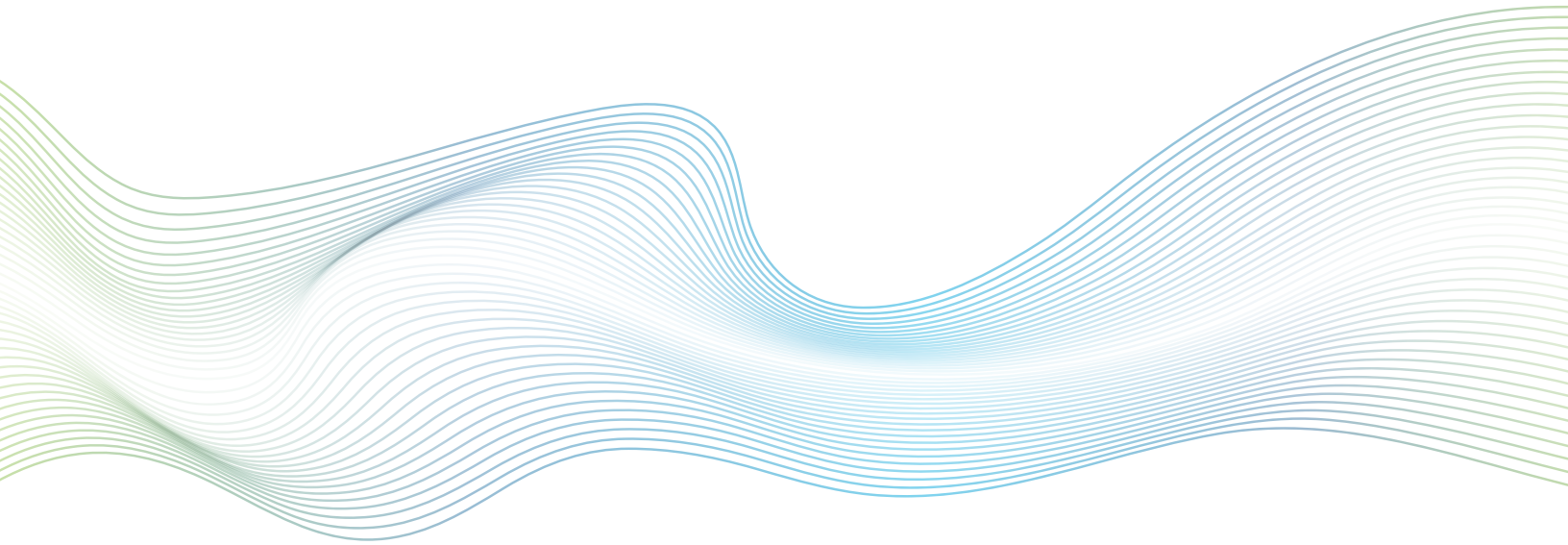
Application Protocol

SynFire® iN plating protocol and chemical long-term potentiation on MaxWell Biosystems Multi-Well High-Density Microelectrode Array (HD-MEA)



NeuCyte
Translatable Neuroscience

maxwell
BIOSYSTEMS
Every Cell Has A Story To Tell

REQUIRED MATERIALS

Item	Supplier	Catalog Number
NeuCyte Seeding Media and Supplement	NeuCyte	2001-20 and 2001s-20
Neucyte Short-Term Media and Supplement	NeuCyte	2002-40 and 2002s-40
Neucyte Long-Term Media and Supplement	NeuCyte	2003-120 and 2003s-120
MaxTwo 6-Well Plate (with Lid)	MaxWell Biosystems AG	MX2-S-6W
Ethanol 70%	Multiple Vendors	
Cell Culture Grade Sterile Deionized Water	Multiple Vendors	
Sterile Syringe Filter (0.22 µm pore)	Multiple Vendors	
Terg-a-zyme	Sigma-Aldrich	Z273287
Breathe-Easy® Sealing Membrane	Sigma-Aldrich	Z380059
Borate Buffer 20X	ThermoFisher Scientific	28341
Poly-Ethyleneimine (PEI)	Sigma-Aldrich	P3143
Laminin	Sigma-Aldrich	L2020
Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12)	ThermoFisher Scientific	1132-033
Neurobasal A	ThermoFisher Scientific	10888-022
10% Bovine Serum Albumin (BSA)	Miltenyi Biotec	130-091-376
Matrigel	Corning	354277
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	D2650
Forskolin	Tocris	1099
Rolipram	Tocris	0905

Application Protocol

Step 1
Day -3
Terg-a-zyme treatment, sterilization, and pre-conditioning

Step 2
Day -1
PEI coating

Step 3
Day0
Laminin coating and plating cells

Step 4
Day 1
50% media change to Short-Term Media

Step 5
Day3
50% media change with Short-Term Media

Step 6
Day 5
50% media change with Short-Term Media

Step 7
Day7
Record neuronal activty and
50% media change with Long-Term Media

Step 8
Day9
50% media change with Long-term Media

Step 9
Day 14
Record neuronal activty

Step 10
Day 21
Record neuronal activty

MaxTwo 6-Well Plate Surface Preparation

Note: The following surface preparation steps are crucial for the hydrophilicity of the chip surface.

1. Prepare a 1%-Terg-a-zyme-solution (10 g/l) in deionized water. Always use a fresh 1%-Terg-a-zyme solution.
2. Add 2 ml of a 1%-Terg-a-zyme-solution into each well of the MaxTwo 6-Well Plate and incubate at room temperature for 2 hours.
3. Aspirate the 1%-Terg-a-zyme-solution and rinse each well of the MaxTwo 6-Well Plate three times with deionized water. To ensure that the 1%-Terg-a-zyme-solution is washed out completely, completely aspirate any remaining deionized water. Make sure to avoid touching the electrode surface.

MaxTwo 6-Well Plate Sterilization and Pre-Conditioning

1. For sterilization: Spray the MaxTwo 6-Well Plate front and back thoroughly with 70% ethanol (see Figure 1).
2. Fill each well and each compartment of the MaxTwo 6-Well Plate with 70% ethanol (see Figures 2 and 3).
3. Transfer the MaxTwo 6-Well Plate to a biological safety cabinet.
4. Remove by aspiration the 70% ethanol from the wells and compartments after 30 minutes.
5. Wash each well of the MaxTwo 6-Well Plate three times with sterile deionized water.
6. Aspirate the water with a vacuum pump and dry the bottom of the MaxTwo 6-Well Plate. Note: Make sure that the bottom of the MaxTwo 6-Well Plate is dry.
7. For pre-conditioning: Fill each well of the MaxTwo 6-Well Plate with 1.2 ml of NeuCyte Seeding Media.
8. Cover the MaxTwo 6-Well Plate with a Breathe-Easy® sealing membrane, and then place the Lid on top.
9. Incubate the MaxTwo 6-Well Plate inside the 5% CO^2 incubator at 37°C, relative humidity (RH) >95%, for 2 days.

10. Before cell plating, aspirate the cell culture media from the MaxTwo 6-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.

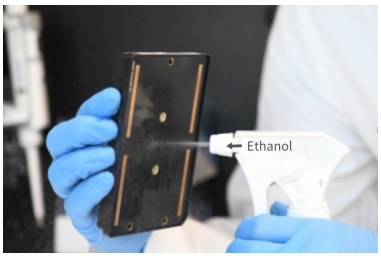


Figure 1: Spraying MaxTwo Multi-Well Plate with 70% Ethanol

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

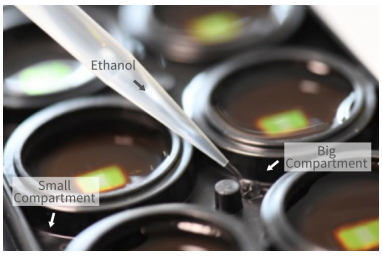


Figure 3: Filling Compartments between Wells with 70% Ethanol

Preparation of Primary Coating with PEI Solution

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 centrifuge tube and allow 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%.
4. For sterilization before use, filter through a 0.22 µm filter unit.

Procedure for PEI Primary Coating

1. Add 50 µl of the PEI Primary Coating Solution to each well of the MaxTwo 6-Well Plate making sure to cover the entire electrode array.
    a. Note: Gently pipette the coating solution onto the center of each electrode array. Avoid touching the surface with the pipette tip.
2. Cover the MaxTwo 6-Well Plate with a Breathe-Easy® sealing membrane and then place the Lid on top.
3. Incubate the MaxTwo 6-Well Plate inside the 5% CO^2 incubator at 37°C, RH >95% for 1 hour.
4. Aspirate the PEI Primary Coating Solution completely.
5. Wash each well of the MaxTwo 6-Well Plate three times with 1 ml sterile deionized water.
6. Aspirate the sterile deionized water with a vacuum pump. Let the MaxTwo 6-Well Plate dry inside the biological safety cabinet for a minimum of 60 minutes.

Procedure for Laminin Secondary Coating

1. Prepare a 20 µg/ml laminin solution by diluting the stock (100 µg aliquots) in sterile deionized water.
2. Add the 50 µl of the Laminin Secondary Coating Solution to each well of the MaxTwo 6-Well Plate.
3. Cover the MaxTwo 6-Well Plate with a Breathe-Easy® sealing membrane and then place the Lid on top.
4. Incubate the MaxTwo 6-Well Plate inside the 5% CO^2 incubator at 37°C, RH >95% for 1 hour.

Thawing and Counting Cells

1. Prepare all media prior to defrosting cells.

MEDIA TO PREPARE:

1) Neurobasal A + 1% BSA
2) NeuCyte Seeding Media

2. Prepare labeled 15 ml tubes. One tube per cell type (glutamatergic, GABAergic, astrocytes) with 3 ml (per cryovial being defrosted) of Neurobasal + 1% BSA will be needed.
3. For example, if defrosting 2 vials of glutamatergic neurons, prepare a tube with 6 ml Neurobasal + 1% BSA. Prepare one tube per plating condition to combine cells for co-culture (See Table 1).

Table 1. Number of cells plated in whole well plating protocol. N.B. Cell number and media volume calculations include a 10% scale-up.

Cell Type	Cells per well	Total cell number for 6 wells
Glutamatergic Neurons	300,000	$1.98x10^6$
GABAergic Neurons	130,000	$0.86x10^6$
Astrocytes	300,000	$1.98x10^6$

4. To defrost cells, place frozen cryovial in a 37°C water bath for 3 minutes with gentle agitation, ensuring the lid remains above the waterline. After 3 minutes, cells should be mostly defrosted with only a small ice crystal remaining.
5. Spray cryovial with 70% EtOH and place in biosafety cabinet.
6. Add dropwise 1 ml of Neurobasal A + 1% BSA into the cryovial.

7. Once added, carefully resuspend cells by pipetting up and down a few times and transfering the 2 ml from the cryovials and adding the cells to the 3 ml Neurobasal A + 1% BSA (total of 5 ml). Distribute cells evenly throughout the 5 ml by gentle pipetting using a 5 ml serological pipette.
8. To count cells, combine 10 µl Trypan blue stain with 10 µl cell suspension. Count cells using a hemocytometer. Perform a total of 4 independent counts and average the 4 counts to obtain the cell concentration. If it is projected to take longer than 1 hour to plate cells, keep cells on ice to reduce cell death.
9. Repeat for all cell types.

Preparing Cells for Co-Culture and Plating Onto MaxWell Biosystems MaxTwo 6-Well Plate

1. Based on the cell count, calculate the volume of cell suspension needed for each cell type.
2. In a new sterile conical tube, add the required volume for each cell type. Each tube should contain a mixture of glutamatergic neurons, GABAergic neurons, and astrocytes in a 7:3:7 ratio respectively.
3. Once all cell types have been added to the 15 ml conical tube, centrifuge tube at 300xG for 3 minutes.
4. Aspirate media and resuspend cells in NeuCyte Seeding Media with the required volume (see Table 3).
5. Aspirate the Laminin Secondary Coating Solution on the MaxTwo 6-Well Plate (do not wash after removal of the Laminin Secondary Coating Solution) and work quickly to seed cells to prevent the laminin coating from drying. Seed the required volume and cells per well.

Table 2. Final cell numbers and resuspension volumes. N.B. Cell number and media volume calculations include a 10% scale-up.

Cell Type	**Whole Well**
Glutamatergic Neurons	$1.98x10^6$ cells
GABAergic Neurons	$0.86x10^6$ cells
Astrocytes	$1.98x10^6$ cells
Resuspension Volume per 6 wells on 6 well plate	330 µl
Volume to plate per well	50 µl

Plating NeuCyte SynFire® iNs on MaxWell Biosystems MaxTwo 6-Well Plates

FOLLOW MAXWELL GUIDELINES FOR PLATING:

1. Add 50 µl of a cell suspension to each well of the MaxTwo 6-Well Plate in the center of the electrode array.
2. Cover the MaxTwo 6-Well Plate with a Breathe-Easy® sealing membrane and place the lid on top.
3. Incubate the MaxTwo 6-Well Plate inside a 5% CO^2 incubator at 37°C, RH >95% overnight.
    a. Note: During cell culture maintenance in the incubator, keep the MaxTwo 6-Well Plate covered with the Breathe-Easy® sealing membrane to prevent evaporation.
4. The following day, carefully fill each well of the MaxTwo 6-Well Plate with 1.15 ml NeuCyte Short-Term Media for a total of 1.2 ml culture media per well. Gently add media by pipetting liquid on the side of the well when adding culture media.

NEUCYTE SYNFIRE IN MAINTENANCE

1. Perform half media changes using NeuCyte Short-Term Media every two days (i.e., Monday, Wednesday, and Friday).
2. At Day 7 post plating, perform the media change using the NeuCyte Long-Term Media.
3. Every Friday, check the volume of media in each well, and top off with fresh media accordingly to ensure the 1.2 ml total volume is maintained.

MAXWELL BIOSYSTEMS' MAXTWO 6-WELL PLATE CELL ELECTRICAL ACTIVITY RECORDINGS

1. It is recommended to perform the first recording 48 hours after media change on Day 7 post-plating. However, this may vary depending on assay design.
2. The recording session is recommended to be between 4 to 24 hours after any further media changes.
3. A fixed time duration between media change and recording is also recommended.
4. Before inserting the MaxTwo 6-Well Plate into the MaxTwo Mainframe, turn on the device and the gas supply 30 minutes prior to performing readings.
5. After inserting the MaxTwo 6-Well Plate into the MaxTwo Mainframe, it is recommended to wait 10 minutes before starting your recording session.

Chemical Long-Term Potentiation (cLTP)

Step 1 2 days befor cLTP
Media change. Ensure 2 ml media final volume

Step 2 Baseline recording

Step 3 Compound treatment

Step 4 30 min post treatment recording

Step 5 Wash and replace conditioned media

Step 6 4 hour post treatment recording

Step 7 24 hour post treatment recording

Step 8 48 hour post treatment recording

Compounds used for cLTP include Forskolin (50 µM) and Rolipram (0.1 µM). Make up a 500X stock solution containing both compounds in DMSO. This stock solution can be aliquoted and stored at -20°C.

1. The media change prior to addition of cLTP compounds, remove 0.2 ml media (ensure there is 1 ml media left in the well). Add 1 ml fresh media, to have a total of 2 ml media in the well (resulting in a 50% media change).
2. On day of compound treatment, remove 1.5 ml of media and save (conditioned media) from each well leaving 0.5 ml media in each well.
3. In a fresh tube, add 0.5 ml conditioned media per well treated with cLTP compounds (for example, if treating 3 wells with cLTP compounds, add 1.5 ml conditioned media in a tube). At the same time, in a separate tube collect the same volume of media per well for DMSO control treatments.
4. To prepare a 2X solution of cLTP compounds, add either 6 µl of the 500X cLTP compounds, or 6 µl DMSO to the 1.5 ml conditioned media to make a 2X solution of cLTP compounds.
5. Perform a baseline recording (Activity Scan Assay, Network Assay, and AxonTracking Assay) on the MaxTwo Multi-Well HD-MEA System prior to compound treatment.
6. After baseline recording, add 500 µl 2X cLTP solution to each well to have a 1X cLTP or DMSO solution in the well.
7. After 30 minutes of compound treatment, perform another recording (same as the baseline recordings).
8. Once these recordings have finished, remove the media from each well and wash 3 times with 2 ml warm PBS.
9. After the final wash, add 1 ml warmed conditioned media back to the plate. Perform another recording 4 hours after cLTP treatment. Following this recording, add 1 ml of fresh media to each well and place back in the cell culture incubator.
10. Perform recordings again after 24, 48, and 72 hours post cLTP treatment.