Human iPSC-Derived Cerebral Organoid differentiation Kit

Human iPSC-Derived Cerebral Organoid Differentiation Kit
Cat. No. : RIPO-BWM001K

Product Description

Human iPSC-Derived Cerebral Organoid Differentiation Kit (Ca. No. RIPO-BWM001K) allows hESC or hiPSC to differentiate into cerebral organoids. Cerebral organoids are three-dimensional in vitro models with a cellular composition and structural organization that is reassembles to the human cerebral regions. This kit can produce 96 cerebral organoids in three steps. Differentiation was carried out by forming EBs from PSC at an ultra-low attachment U shape 96 well plate, and then changing the medium according to the instructions. Organoids generated using Human iPSC-Derived Cerebral Organoid Differentiation Kit (Ca. No. RIPO-BWM001K) feature various types of neurons (including TH positive neurons) and glia cells (including OLIG2 and IBA1 positive cells). These cerebral organoids show spontaneous electrophysiological activity and response to a-syn PFFs induced toxicity, representing the functionality of the organoids.

Product Specification

The basic medium of this differentiation kit is a serum-free, well-defined medium with minimal batch variation to which differentiation factors are added. This medium does not contain antibiotics, the addition of which may affect cerebral organoid differentiation.

Product Information

Name	Component #	Size	Storage	Shelf Life
Basal Medium A	RIPO-BWM001K -C01	22.5ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement A-1	RIPO-BWM001K-1 -C01	2ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement A-2	RIPO-BWM001K-1 -C02	0.5ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium B	RIPO-BWM001K -C02	22.5ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement B-1	RIPO-BWM001K-1 -C03	2ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement B-2	RIPO-BWM001K-1 -C04	0.5ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Basal Medium C	RIPO-BWM001K -C03	90ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement C-1	RIPO-BWM001K-1 -C05	8ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement C-2	RIPO-BWM001K-1 -C06	2ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label

Materials Required but Not Included

- mTeSR Plus (STEMCELL Technologies, # 100-0276)
- Gentle Cell Dissociation Reagent (STEMCELL Technologies, # 100-0485)
- D-PBS (Without Ca++ and Mg++)
- Ultra-Low Adherent 96 Well Plate
- Ultra-Low Adherent 6 Well plate
- Matrigel (Corning, #354277)
- Hemocytometer
- Trypan blue
- Y-27632 (Mce, #HY-10071)

Equipment Required

- Incubator (37°C, 5% CO_2)
- Low-speed centrifuge with a swinging bucket rotor with an adaptor for plate holders
- Orbital shaker
- Biosafety cabinet

Protocol Diagram

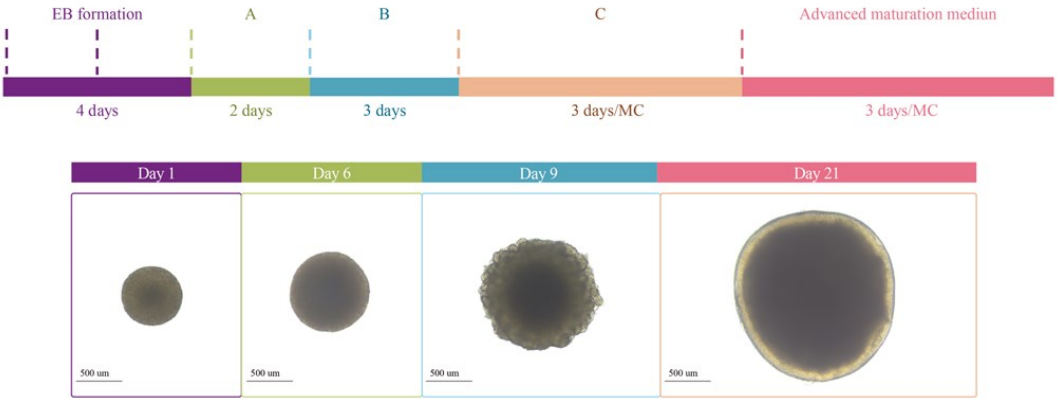


Figure 1. Protocol Diagram of cerebral organoid differentiation.

The color differs each component of differentiation kit. The dashed line represents the time for medium changes. Morphology of Cerebral organoid at each stage of differentiation could be observed.

Preparation of Media

Use sterile technique when performing the following manipulation.

Medium	Component	Volume	IN-USE STORAGE/STABILITY
Medium A (25ml)	Basal Medium A	22.5ml	Mix completely the Basal Medium A, Supplement A-1 and Supplement A-2 to get Medium A. (Note, Mix Basal Medium A with Supplement A-2 first and then mixing with Supplement A-1) Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement A-1	2ml	
	Supplement A-2	0.5ml	
Medium B (25ml)	Basal Medium B	22.5ml	Mix completely the Basal Medium B, Supplement B-1 and Supplement B-2 to get Medium B. (Note, Mix Basal Medium B with Supplement B-2 first and then mixing with Supplement B-1) Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement B-1	2ml	
	Supplement B-2	0.5ml	
Medium C (100ml)	Basal Medium C	90ml	Mix completely the Basal Medium C Supplement C-1 and Supplement C-2 to get Medium C. (Note, Mix Basal Medium C with Supplement C-2 first and then mixing with Supplement C-1) Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement C-1	8ml	
	Supplement C-2	22ml	

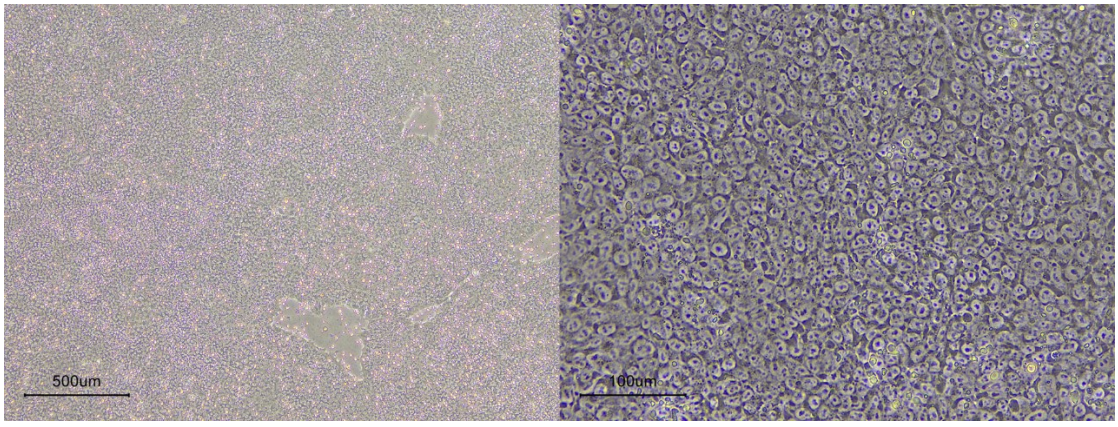
Note: Please do not heat the complete medium (mixture of basal medium and supplement). Use it directly as cold as 2-8 °C.

Directions for Use

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols.

Note: Before Cerebral organoid culturing, please make sure that the culture system you use is mTeSR-based. If your culture system is not mTeSR, please make sure that you have transferred your cells to the mTeSR system for at least 4 passages.



The ips cell quality should be verified to exhibit distinct borders, tight packing and less than 10% differentiation with respect to their colonies surface area.

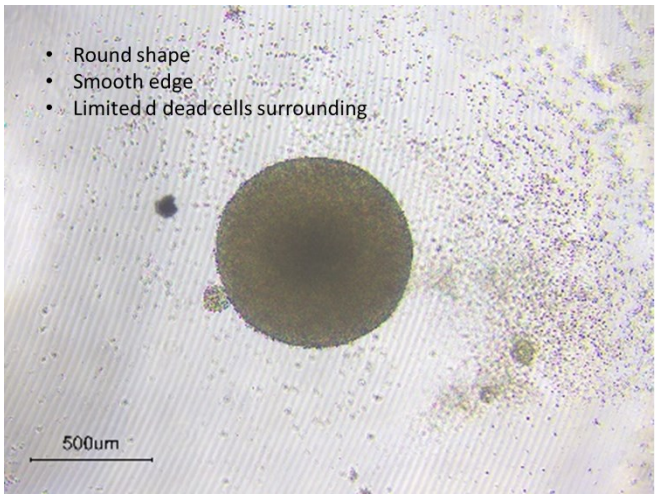
EB Formation

1. Rince hPSC cultures with pre-warmed D-PBS (Without Ca++ and Mg++) 3 times.
2. Aspirate and replace with 2 mL (for 1 well of 6well plate) of Gentle Cell Dissociation Reagent.
3. Incubate about 7-10 minutes for digestion of iPSCs to single cells.

Note: Incubation time may vary when using different cell lines or different cell dissociation reagents.

4. Pipet cells for obtaining single cells, transfer to the tube and centrifuge at 300g, 4 °C for 5 minutes.
5. Remove the supernatant and add 1 ml mTeSR Plus to resuspend cells.
6. Count cells using Trypan Blue and a hemocytometer.
7. Transfer appropriate number of cells into mTeSR Plus with 10 uM Y-27632 to acquire final concentration of 7,500 cells/100 µL.
8. Add 100 µL of cell suspension into each well of a 96-well round-bottom ultra-low attachment plate.
9. Centrifuge the ultra-low attachment plate seeded with cells at 300g, 4 °C for 5 minutes.
10. Incubate the plate at 37°C, 5% CO_2 for 24h to formation of embryoid bodies.
11. Count the day after 24h incubation as day 0. Verify the formed EBs at day 0 have a size about 350 to 500 µm and a smooth round edge.
12. Continue to incubate for 48h.
13. At day 2, add 100 ul of mTeSR Plus (no Y-27632) into each well of EB formed well. Continue to incubate for 48 h.

EB Validation



Cerebral Organoid Differentiation

1. At day 4, remove all the medium in the wells. Add 200µl of Medium A at each well and incubate at 37°C, 5% CO_2 for 48h.
2. At day 6, remove all Medium A in each well and add 200µl of Medium B in each well, incubate at 37°C, 5% CO_2 for 72 h.

   Optional step: mix 1% of Matrigel (Corning, #354277) with Medium B for B stage organoid culture, will help the organoid growth faster. If doing this step, please make sure the 1% Matrigel mixed Medium B is kept at 4℃ in whole experiment step.

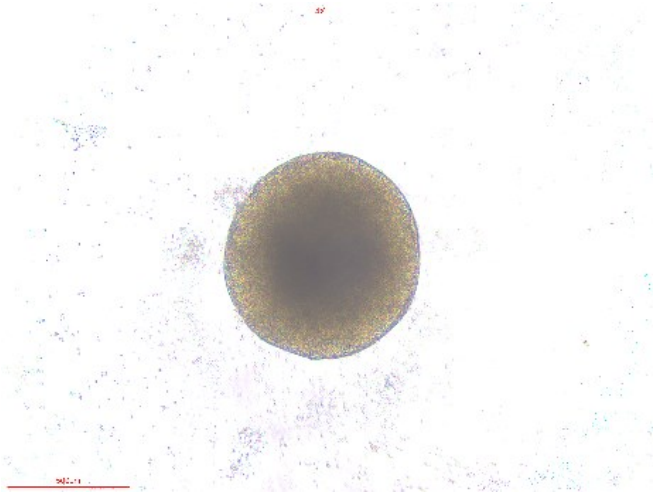
3. At day 9, transfer all the organoids into Ultra-Low Adherent 6 Well plate with in maximum 24 organoids per well, add 5 ml medium C per well.
4. Put the plate on an orbital shaker, set the shaker at 100 rpm and place it into the incubator, incubate at 37°C, 5% CO_2.
5. Full medium change of medium C every 3 days.

Cerebral Organoid maturation

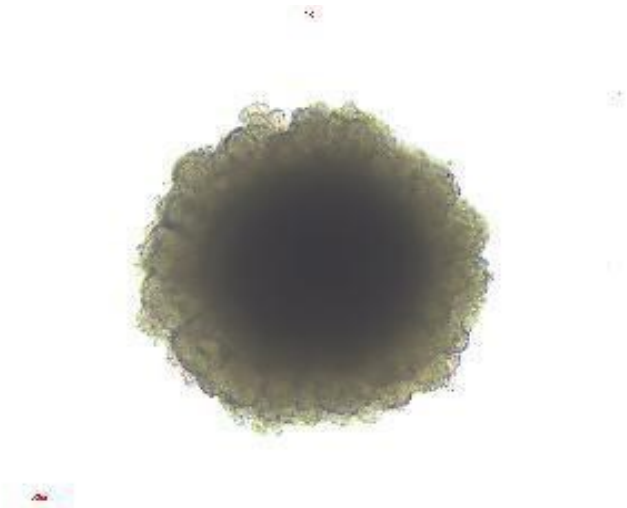
1. After 15 days of culture in Medium C (5 medium change in total), aspirate and add 5 ml of Medium MM (not included in this kit, Cat: RIPO-BWM003) per well.
2. Full medium change of medium MM every 3 days.

Differentiation Validation

Morphology at day 5



Morphology at day 8



Related Products

For further maturation of cerebral organoid, please use Human iPSC-Derived Cerebral Organoid Maturation and Maintenance Kit (Cat: RIPO-BWM003).

Product	Cat. No.
Human iPSC-Derived Cerebral Organoid Maturation and Maintenance Kit	**RIPO-BWM003**

Validation Data of Cerebral Organoids

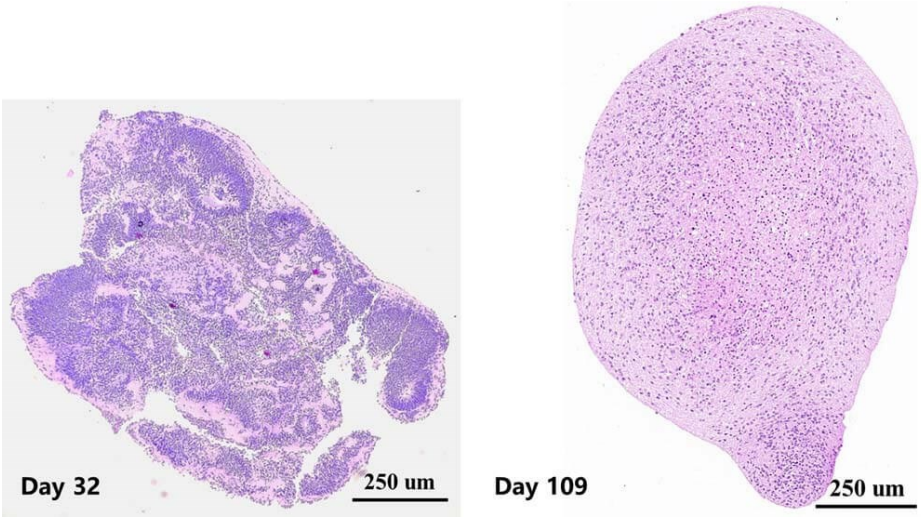


Figure 2. H&E staining of Cerebral Organoids

Left: Early-stage cerebral organoid show rosette-like structures (neural stem cells), which become smaller as organoids develop. Right: Day 109 cerebral organoids show uniform morphology and show no dead core inside.

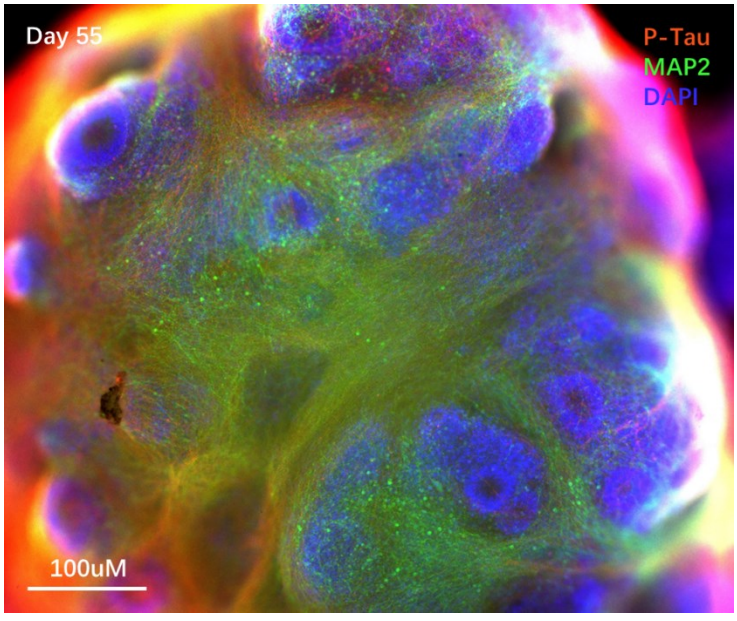


Figure 3. Immunostaining of Day 55 Cerebral Organoids

Presence of MAP2 positive cells and positive cells (Mature neuron marker). Presence of Phosphorylated Tau protein.

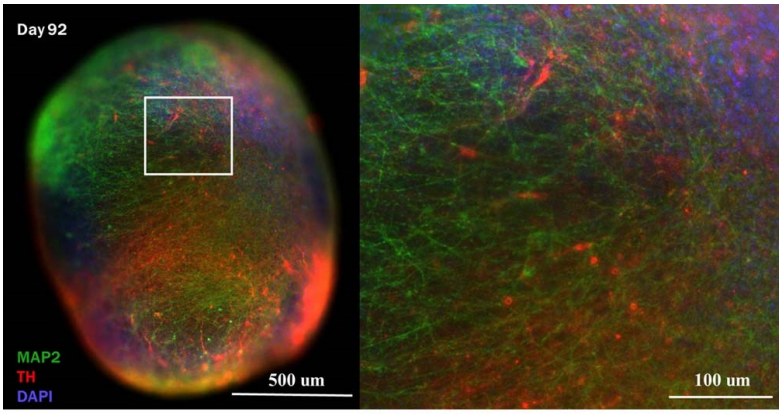


Figure 4. Immunostaining of day 92 Cerebral Organoids

Presence of TH and MAP2 positive neurons in day 92 cerebral organoid. TH: used as cell marker of dopaminergic neurons. MAP2: mature neuron cell marker.

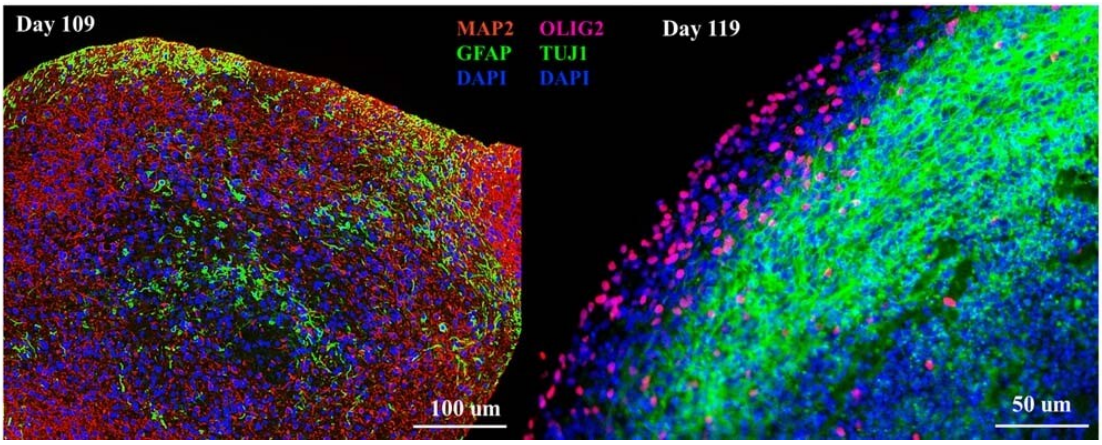


Figure 5. Immunostaining of Cerebral Organoids show expression of glia cell markers.

Left: Presence of GFAP positive cells at day 109 cultured cerebral organoid. Right: Presence of OLIG2 positive cells at day 119 cultured cerebral organoid. GFAP: marker for astrocyte. OLIG2: marker for oligodendrocyte.

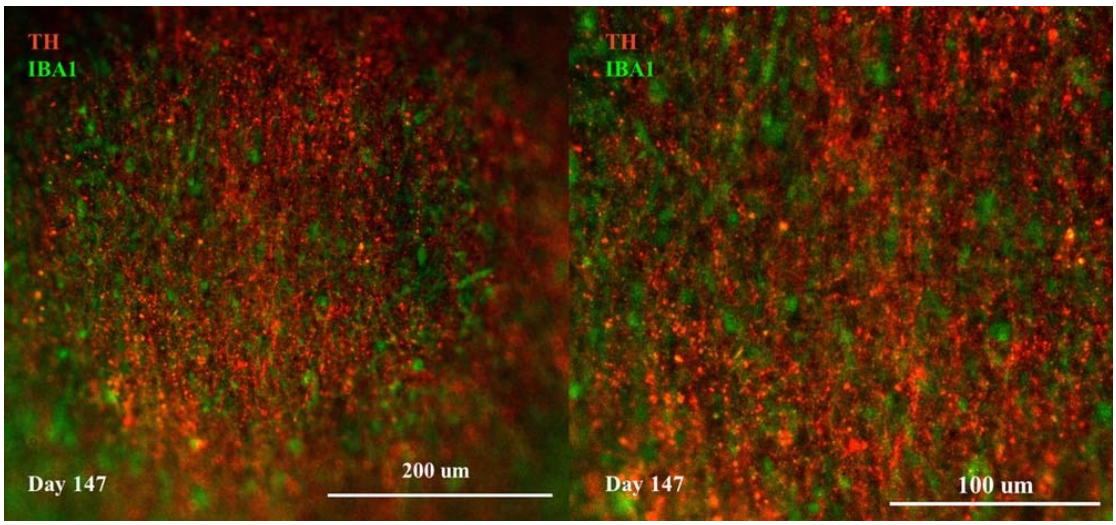


Figure 6. Immunostaining of Cerebral Organoids show expression of IBA1 marker.

Presence of TH and IBA1 positive cell in day 147 cultured cerebral organoid. IBA1: cell marker for microglia.

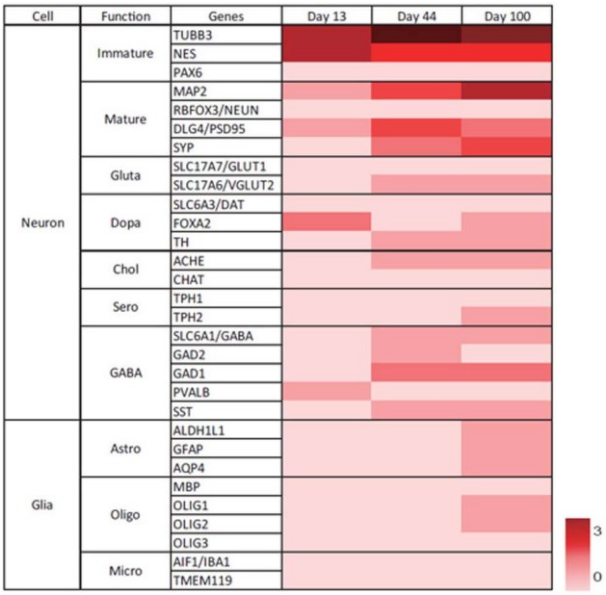


Figure 7. RNA-Seq analysis of cerebral organoids at different stages

RNA-Seq analysis of cerebral organoid of day 13, day 44 and day 100, showing the expression of many markers for glutamatergic, dopaminergic, cholinergic, serotonergic and GABAergic neuron. In addition, glia cell markers were also expressed.

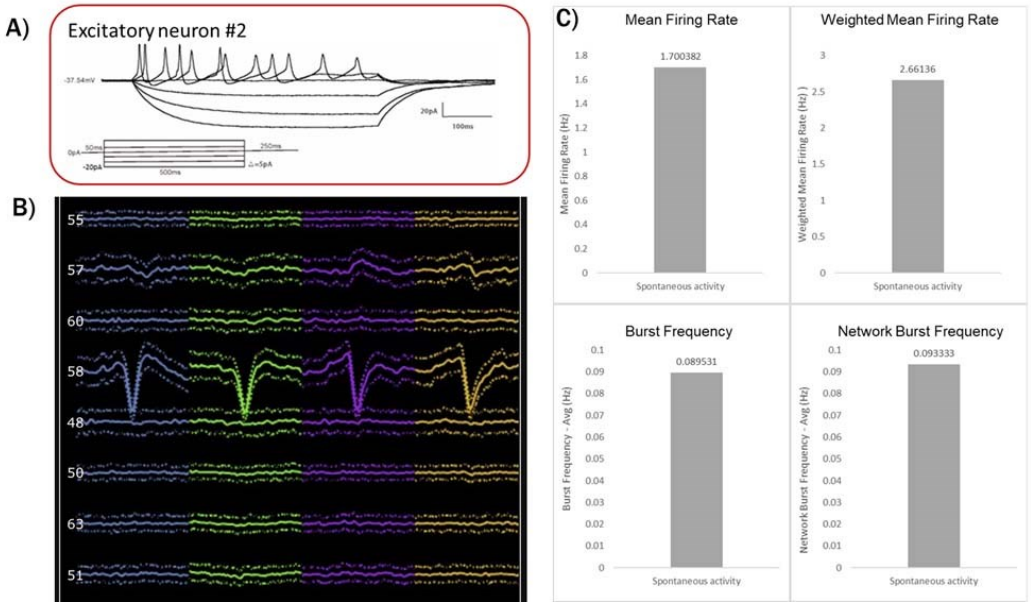


Figure 8. Electrophysiology analysis of cerebral organoids

A: patch-clamp recording of excitatory neurons sectioned from cerebral organoids at day 102. Excitatory neuron showed stable response to step injection currents. B: Recording of cerebral organoid (day 60) using silicon probe. Neurons showed spontaneous activities and different waveforms. C: MEA recording indicated spontaneous bursting activities for cerebral organoid (day 86)."