Human iPSC-Derived Cerebral Organoid Maturation and Maintenance Kit

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

Product Description

Cerebral organoids are three-dimensional in vitro models with a cellular composition and structural organization that is representative to the human cerebral regions. Human iPSC-Derived Cerebral Organoid maintenance Kit allows the maturation and the long-term maintenance of cerebral 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 MM	RIPO-BWM003 -C01	225ml	4 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement MM-1	RIPO-BWM003-1 -C01	20ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label
Supplement MM-2	RIPO-BWM003-1 -C02	5ml	-20 °C	Stable for 1 years from date of manufacture (MFG) on label

Materials & Equipment Required

- Ultra-Low Adherent 6 Well plate（Cat. CP-21）
- Incubator (37°C, 5% CO_2)
- Orbital shaker (20 mm shaking diameter)
- 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 MM (250ml)	Basal Medium MM	225ml	Mix completely the Basal Medium MM, Supplement MM-1 and Supplement MM-2 to get Medium MM. (Note, Mix Basal Medium MM with Supplement MM-2 first and then mixing with Supplement MM-1) Store at 2 - 8°C for up to 2 weeks or aliquot as desired.
	Supplement MM-1	20ml	
	Supplement MM-2	5ml	

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

Note: Human iPSC-Derived Cerebral Organoid Maturation and Maintenance Kit is used of the maturation and maintenance of differentiated cerebral organoids. Before using this kit, please use Human iPSC-Derived Cerebral Organoid Differentiation Kit (Cat: RIPO-BWM001K) to achieve cerebral organoid differentiation to 24 days.

Cerebral Organoid maturation

1. At the Medium C stage (Human iPSC-Derived Cerebral Organoid Differentiation Kit, Cat: RIPO-BWM001K), transfer the organoids to an ultra-low attachment 6-well plate (Ultra-low attachment surface cell culture plate, 6 wells, Cat: CP-21) with no more than 24 organoids per well. Place the plates on an orbital shaker set to 100 rpm and incubate at 37°C with 5% CO_2.
2. Carefully aspirate Medium C and add 5 ml of maturation and maintenance medium (Medium MM) to each well.
3. Replace the medium with fresh Medium MM every 3 days to support cerebral organoid maturation.

Related Products

For the differentiation of cerebral organoid, please use Human iPSC-Derived Cerebral Organoid

Differentiation Kit (Cat: RIPO-BWM001K).

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

Validation Data of Cerebral Organoids

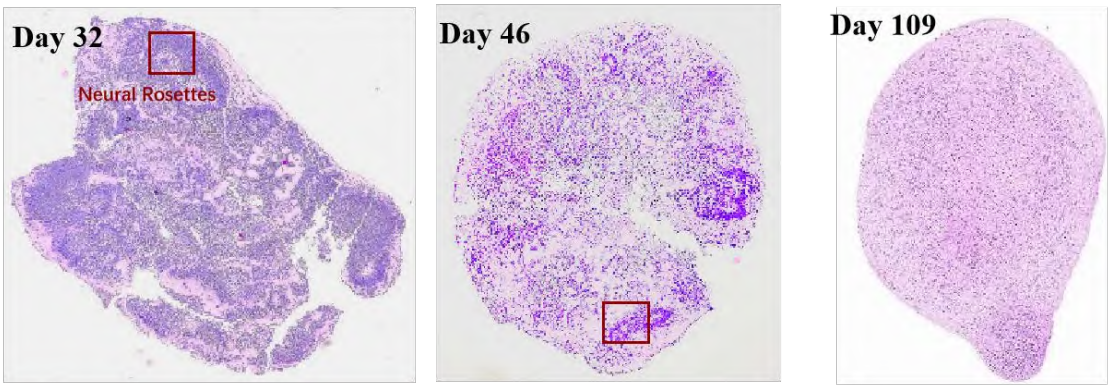


Figure 2. H&E staining of Cerebral Organoids

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.

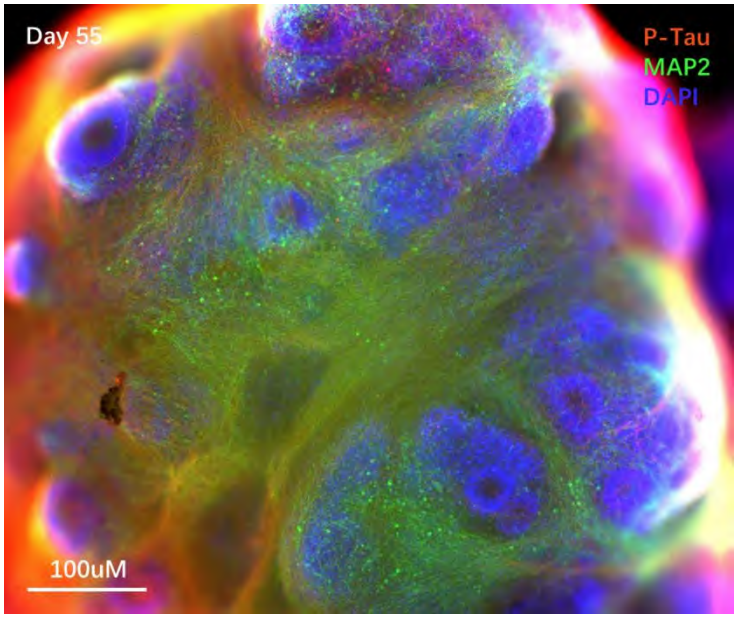


Figure 3. Immunostaining of Day 55 Cerebral Organoids

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

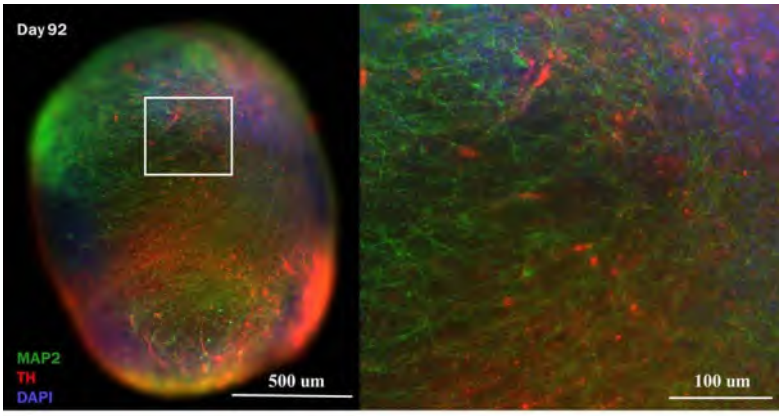


Figure 4. Immunostaining of day 92 Cerebral Organoids

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.

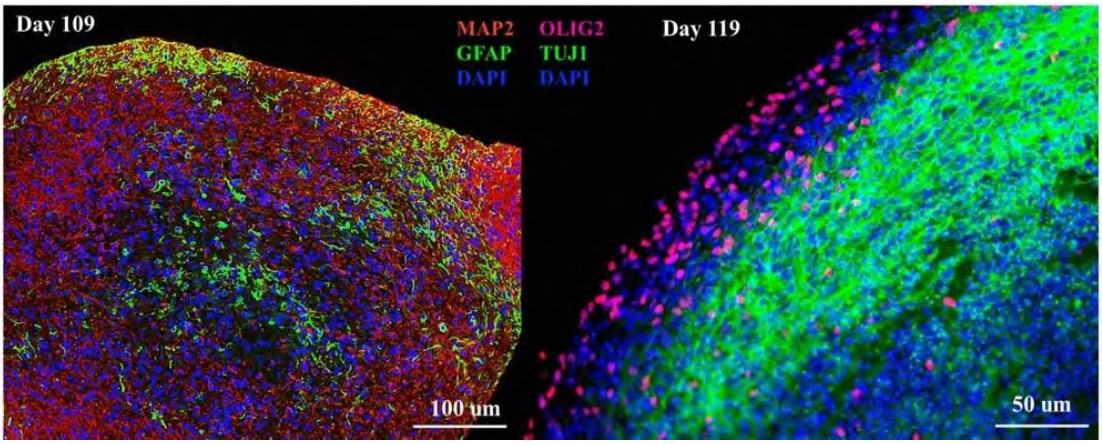


Figure 5. Immunostaining of Cerebral Organoids show expression of glia cell markers.

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.

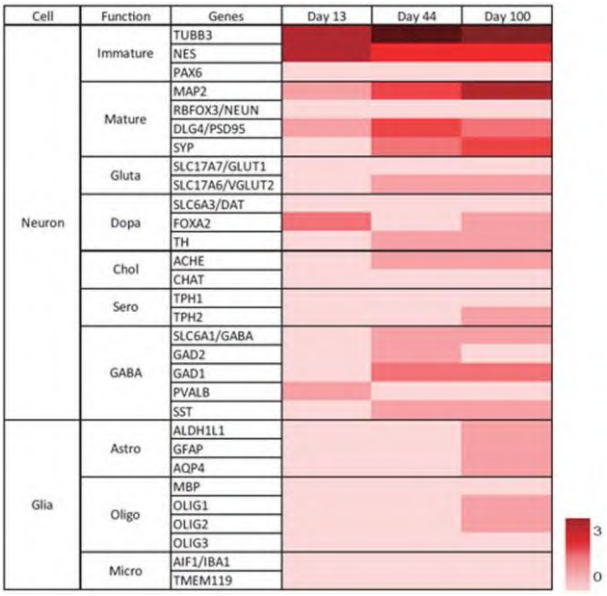


Figure 6. RNA-Seq analysis of cerebral organoids at different stages

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.