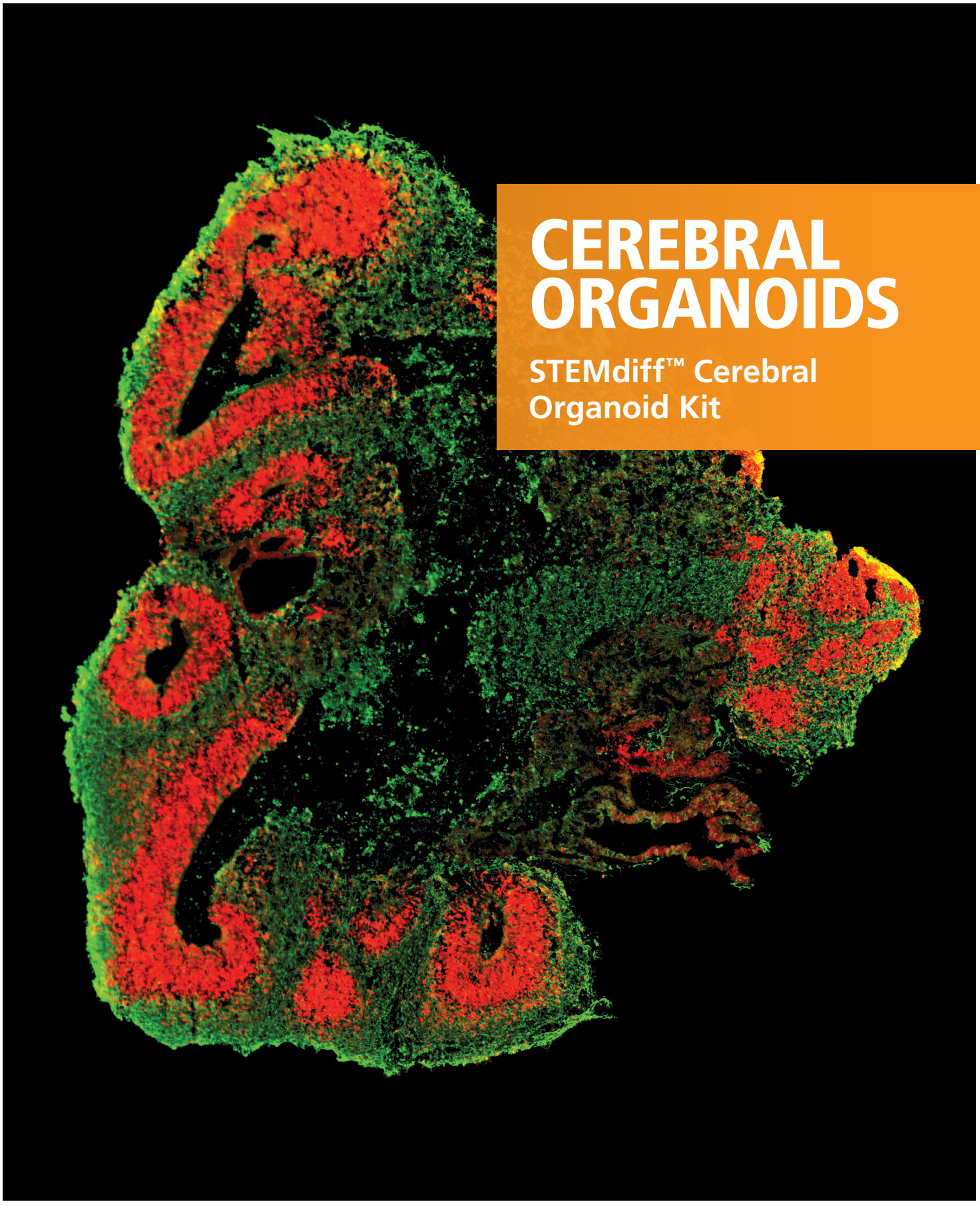

# CEREBRAL ORGANOIDS

## STEMdiff™ Cerebral Organoid Kit

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# Introduction

## For the Culture and Maturation of Human Cerebral Organoids

The metazoan brain is a highly complex and organized structure. Two-dimensional (2D) neural cultures derived from human pluripotent stem cells (hPSCs) are useful models to study the nervous system, but they are limited in their capacity to recapitulate the complex organization of brain tissues.

hPSC-derived cerebral organoids are three-dimensional (3D) in vitro culture systems that recapitulate the developmental processes and organization of the developing human brain.
They provide a physiologically relevant in vitro model for the study of neurological development and disease processes that are unique to the human nervous system. Cerebral organoids have important applications in studying human brain development and neurological disorders such as autism, schizophrenia or brain defects caused by Zika virus infection. The STEMdiff™ Cerebral Organoid Kit is a serum-free culture system that is designed to generate cerebral organoids from human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells.

The kit contains 2 basal media and 5 supplements, which are combined separately to prepare complete media for each of the four distinct stages of organoid formation (Figure 1). Using a simple, optimized protocol based on the formulation published by MA Lancaster and JA Knoblich,[1] the cerebral organoids generated with this kit contain regions that recapitulate the cortical layers observed during in vivo human brain development. These cerebral organoids develop progenitor populations that organize into distinct layers and give rise to mature neurons, matching what is observed in the developing human cortex. The kit is designed to culture organoids for 40 days; for extended periods of organoid culture, the STEMdiff™ Cerebral Organoid Maturation Kit is available separately.

### Why Use the STEMdiff™ Cerebral Organoid Kit?

**PHYSIOLOGICAL.** Three-dimensional in vitro system recapitulates the developmental processes and organization of the developing brain.

**INNOVATIVE.** Serum-free human pluripotent stem cell-based model enables the study of development and disease processes.

**OPTIMIZED.** Formulation is optimized for increased efficiency of organoid formation.

**RELIABLE.** Rigorous raw material screening and extensive quality control testing ensure reproducibility and minimal lot-to-lot variability.

**SIMPLE.** Convenient format and easy-to-use protocol.

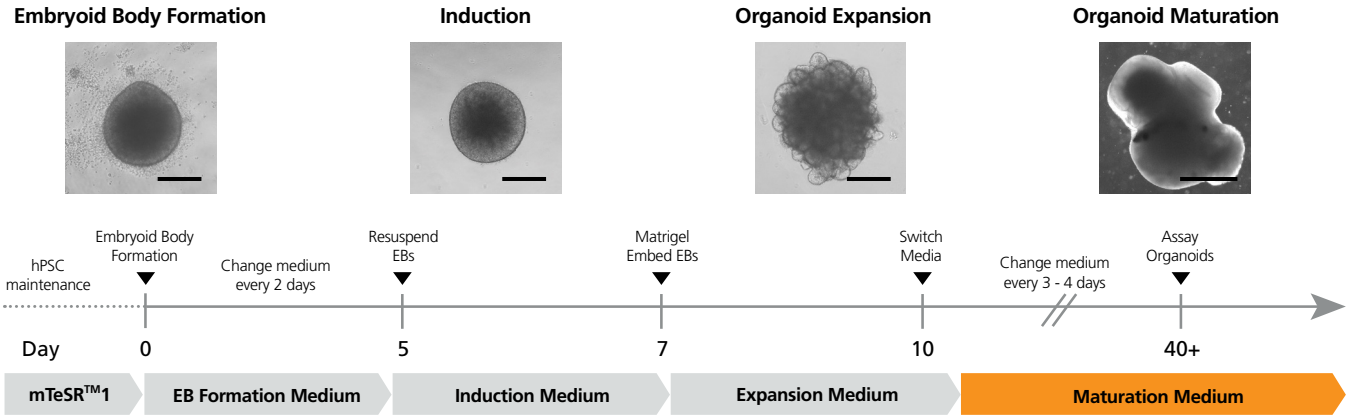


**Figure 1.** Schematic for the STEMdiff™ Cerebral Organoid Kit

Human pluripotent stem cells (either embryonic or induced pluripotent stem cells) maintained in mTeSR1™ were dissociated into single-cell suspensions using Gentle Cell Dissociation Reagent (GCDR) and seeded at a density of 9,000 cells/well in a U-Bottom 96-well Ultra-Low Attachment Plate (Corning®) in EB Formation Medium + 10 µM Rho-Kinase Inhibitor (ROCKi). EBs were fed every 2 days with EB Formation Medium without ROCKi. After 5 days, EBs were transferred to Induction Medium in a 24-well Ultra-Low Attachment Plate (Corning®). EBs were cultured for an additional 2 days and were then embedded in liquid Matrigel® (Growth Factor Reduced, Corning®). They were then transferred to a non-tissue culture treated 6-well plate (12 -16 organoids/well). Embedded organoids were maintained in Expansion Medium for 3 days. On Day 10, organoids were switched to Maturation Medium and cultured on an orbital shaker set at 57-95 RPM (Infors HT). Organoids were fed every 3 – 4 days with Maturation Medium. On Day 40, organoids were processed for analysis by RT-qPCR or immunostaining followed by cryosectioning. Scale bars for EB Formation, Induction and Organoid Expansion =300 µm. Scale bar for Organoid Maturation =1 mm.

# Cerebral Organoid Formation

The STEMdiff™ Cerebral Organoid Kit produces organoids using a simple and reproducible 4-stage protocol (Figure 1). hPSCs cultured in mTeSR™1 or TeSR™-E8™ maintenance medium can be used to generate cerebral organoids. In the Embryoid Body (EB) Formation stage, single-celled aggregates are formed over a period of 5 days (Figure 2, Day 5).

During the Induction stage, the EBs begin to develop translucent and smooth edges over a period of 2 days, indicative of neuroepithelium formation (Figure 2, Day 7). The EBs are then embedded in a Matrigel© droplet in the Expansion stage, where they begin to exhibit a budding morphology after 3 days, which is indicative of neuroepithelium expansion (Figure 2, Day 10). During the Maturation stage of cerebral organoid formation, the organoids become heavier and larger in size over approximately an additional 30 days, reaching a maximum size of 3 - 5 mm in diameter (Figures 3-4). Cerebral organoids are typically assayed at Day 40 for markers of cortical-like regions, at which time they display organization similar to the early developing human cortex.

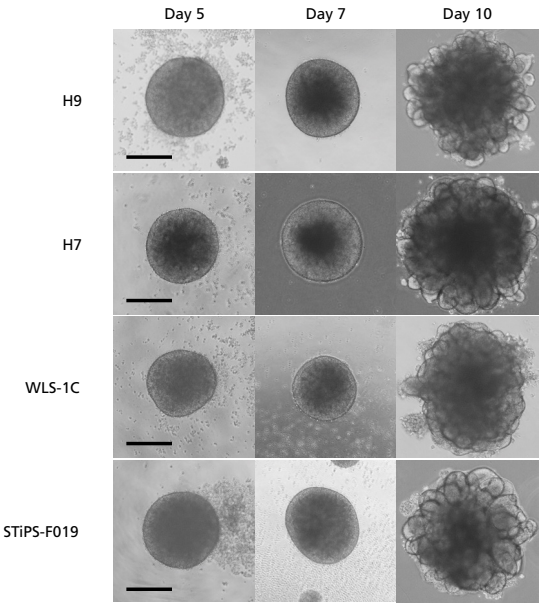


**Figure 2.** The STEMdiff™ Cerebral Organoid Kit Supports EB Formation and Neuroepithelia Expansion for Multiple hPSC Lines

Robust early organoid formation was observed in four representative hPSC lines (2 hESC; H9 and H7, 2 hiPSC; WLS-1C and STiPS-F019). Day 5: Embryoid Body Formation stage; Single-cell suspensions of hPSCs formed spherical aggregates with smooth edges. Day 7: Induction stage; EB edges had smoothened and developed a translucent quality. Day 10: Expansion stage; Matrigel® embedded EBs displayed expanded neuroepithelia evident by bubbling of their surfaces. Over 80% of all embedded organoids exhibited this type of morphology indicative of neuroepithelia expansion (n= 2 per hPSC line, 8 to 24 organoids measured per hPSC line). Scale bar = 300 µm.

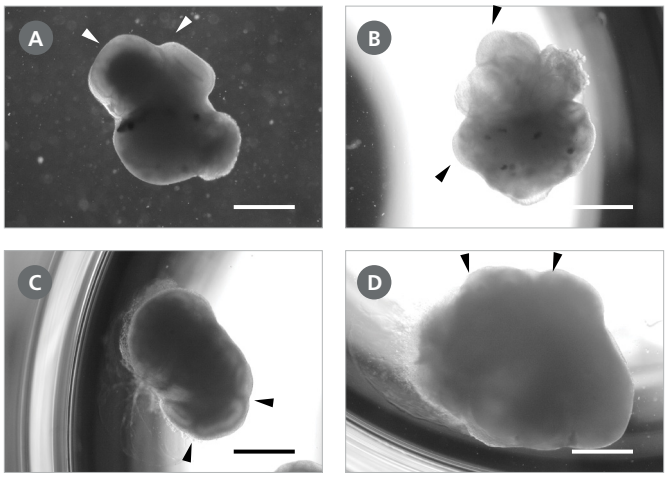


**Figure 3.** Day 40 Cerebral Organoids Grow to Over 1 mm in Diameter and Exhibit Tissue Structures with Dense Cores

Cerebral organoids cultured in Maturation Medium at Day 40 developed thick tissue-like structures and areas that exhibit layering (arrowheads). More than 50% of organoids from each experiment exhibited this type of morphology (n = 2 per cell line, 8 – 24 Organoids measured per hPSC line) (A) H9 (B) H7 (C) WLS-1C (D) STiPS-F019. Scale bar = 1 mm.

**Figure 4.** Day 60 Cerebral Organoids Grow to Over 1 mm in Diameter and Exhibit Tissue Structures with Dense Cores

Size of Day 60 organoids (H9). An American quarter is used for scale. Cerebral organoids are outlined (white dotted circles).

# Cerebral Organoid Characterization

Cerebral organoids are a complex 3D system that recapitulate many features of the developing human brain. In as little as 40 days, cerebral organoids display large optically-dense tissues as observed using phase-contrast microscopy (Figure 6A). When these organoids are cryosectioned and immunostained for markers of the developing human cortex, multiple cortical-like regions are observed (Figure 6B). Similar to what is seen in vivo, PAX6+ apical progenitors are found localized in a ventricular zone-like region, and are distinctly separated from class III β-tubulin+ (TUJ-1) neurons (Figure 6C). A cortical plate-like region is also observed with CTIP2+ and class III β-tubulin+ neurons found next to the PAX6+ apical progenitors (Figure 6D).

Proliferating progenitors labeled by Ki-67 were also found localized along the ventricle, with an additional population of Ki-67+ cells observed in an outer subventricular-zone-like region reminiscent of outer radial glia (Figure 6E-F).

RT-qPCR of whole organoids was used to further validate marker expression, and both markers of neural progenitors (PAX6 and TBR2), and of more mature neurons (TBR1, CTIP2, MAP2, class III β-tubulin) were upregulated in comparison to an undifferentiated hPSC control (Figure 5). A very similar expression profile for these markers was observed from four different cell lines, demonstrating consistency of the STEMdiff™ Cerebral Organoid Kit.

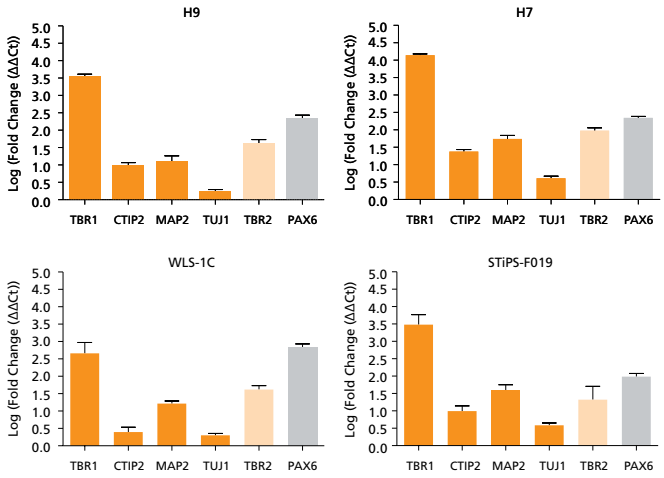


**Figure 5.** Day 40 Cerebral Organoids Contain Progenitor and Neuron Populations that Organize into Distinct Layers

RT-qPCR analysis showed that upregulation of both neural progenitor and mature neuron transcripts as Log (Fold Change ΔΔCt) (Average ± SEM n = 2 per cell line, ≥ 3 organoids per analysis). Data were normalized to 18S/TBP and compared to undifferentiated hPSC control.

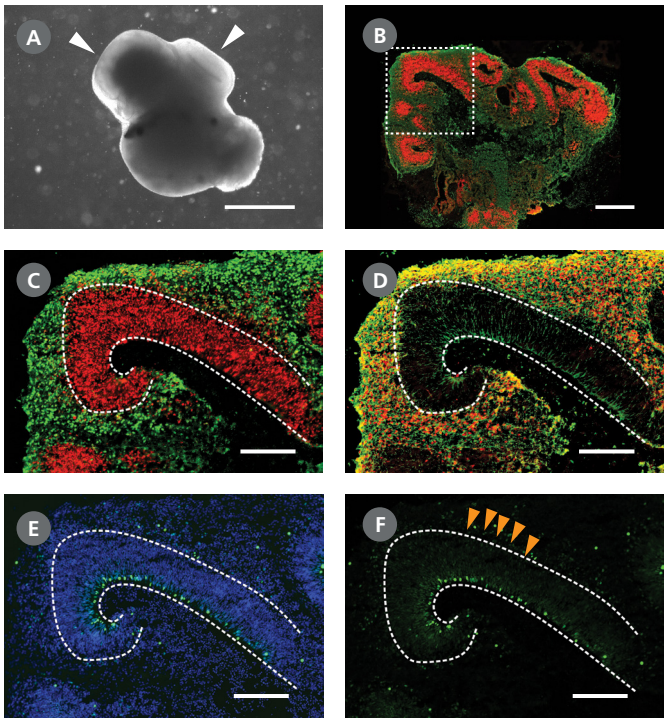


**Figure 6.** Characterization of Cerebral Organoids Generated Using the STEMdiff™ Cerebral Organoid Kit

(A) A representative phase-contrast image of a whole cerebral organoid at Day 40 generated using the STEMdiff™ Cerebral Organoid Kit. Cerebral organoids at this stage are made up of phase-dark structures that may be surrounded by regions of thinner, more translucent structures that display layering (arrowheads). (B) Immunohistological analysis on cryosections of cerebral organoids reveals cortical regions within the organoid labeled by the apical progenitor marker PAX6 (red) and neuronal marker class III β-tubulin (TUJ-1) (green). (C-F) Inset of boxed region from (B). (C) PAX6+ apical progenitors (red, enclosed by dotted line) are localized to a ventricular zone-like region. Class III β-tubulin+ neurons (green) are adjacent to the ventricular zone. (D) CTIP2, a marker of the developing cortical plate, co-localizes with class III β-tubulin+ neurons in a cortical plate-like region. Organization of the layers recapitulates early corticogenesis observed during human brain development. (E) Proliferating progenitor cells labeled by Ki-67 (green) localize along the ventricle, nuclei are counterstained with DAPI (blue). (F) An additional population of Ki-67+ cells is found in an outer subventricular zone-like region (arrowheads). Scale bar = (A) 1 mm, (B) 500 µm and (C-F) 200 µm.

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Whole organoid RNA-sequencing was also performed and used to compare to published data sets.[2] Principal component analysis of hPSC and cerebral organoid transcriptomes (Figure 7) shows that a large majority of the variance (80%) is observed when comparing these samples, while only a modest difference (9%) is observed in comparing cultured organoids and embryonic fetal brain sample (19 post-conceptional weeks). Importantly, the cultured organoids from the Luo 2016[2] data set cluster very close to organoids generated with the STEMdiff™ Cerebral Organoid Kit, indicating a high degree of similarity and confirming that the organoids generated match what is seen in the literature.

Additionally, a heatmap of expression levels for genes associated with synaptic transmission function and neurogenesis show a similar pattern of expression for Day 40 organoids when comparing the published data set and organoids cultured with the STEMdiff™ Cerebral Organoid Kit. Taken together, the data show that the STEMdiff™ Cerebral Organoid Kit generates cerebral organoids that display cortical-like regions, and are relevant models for the early developing human cortex.

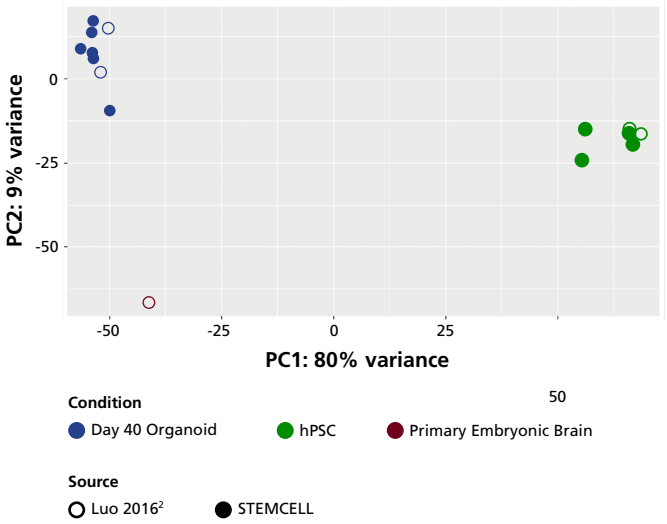


**Figure 7. Organoids Generated with STEMdiff™ Cerebral Organoid Kit Are Similar to Organoids Produced in Literature**

Principal component analysis of hPSC and cerebral organoid transcriptomes. Cerebral organoids generated using the STEMdiff™ Cerebral Organoid Kit (filled blue circles) clustered together, and clustered with previously published cerebral organoids (open blue circles). The first principal component accounted for the majority of variance observed (PC1; 80%) and distinguished the cerebral organoid samples from the hPSCs (green circles). The second principal component accounted for only 9% of the variation, and highlighted the modest difference in gene expression between cultured organoids and primary embryonic fetal brain samples (19 post-conceptional weeks, brown circles).

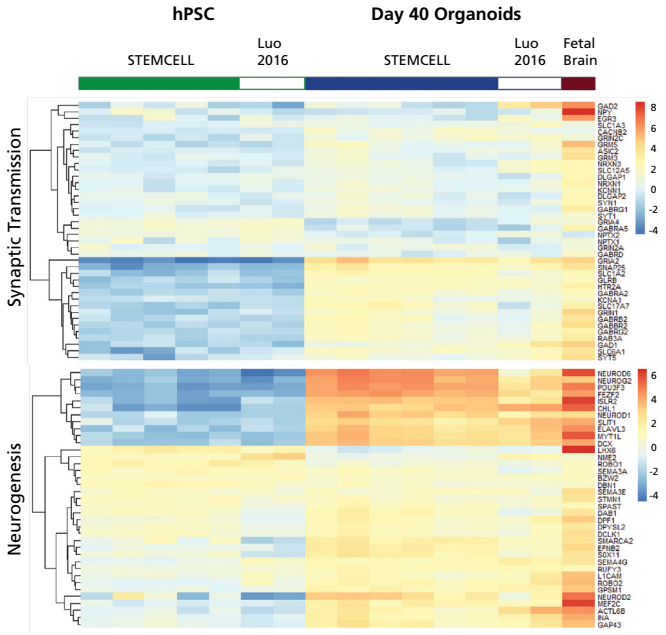


**Figure 8.** RNA-Seq Analysis of Day 40 Organoids Reveals Marker Expression of the Early Developing Human Brain

Heatmap of expression levels for genes associated with synaptic transmission function and neurogenesis in Day 40 organoids. These data show that gene expression of cerebral organoids generated from the STEMdiff™ Cerebral Organoid Kit are similar to published results.

**POSTER BY SERGIU P. PAŞCA**

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**VIDEO**

How to Grow Cerebral Organoids

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Watch our step-by-step video guide to growing cerebral organoids from hPSCs. We'll walk you through all four stages of the protocol: embryoid body formation, induction, expansion, and organoid maturation.

# Performance of STEMdiff™ Cerebral Organoid Kit vs. Unqualified Reagents

The STEMdiff™ Cerebral Organoid Kit offers several advantages over the use of unqualified homemade reagents to culture cerebral organoids. The kit offers a serum-free formulation optimized for increased efficiency of organoid formation, including with hard-to-differentiate cell lines (Figures 9A-B). Rigorous raw material screening and extensive quality control testing ensure reproducibility in the yield of higher quality organoids (Figure 9C-D), and the kit includes all the required components and a simplified protocol that offers greater convenience.

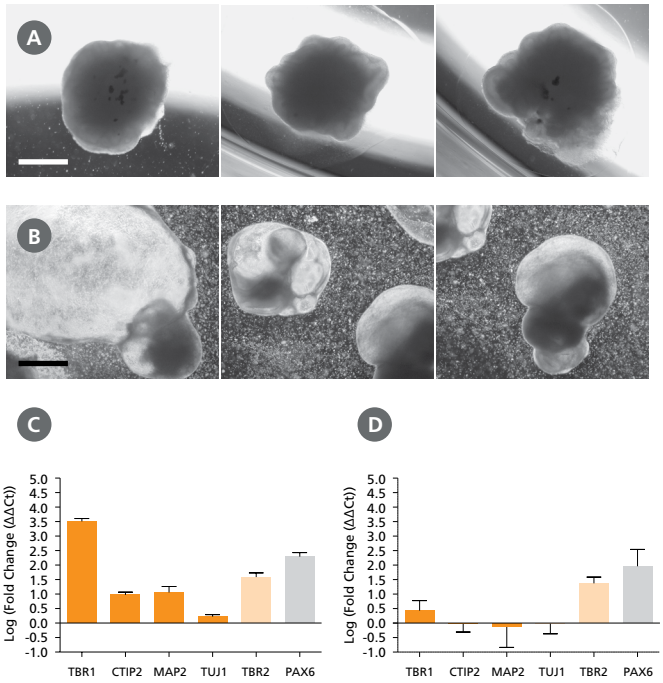


**Figure 9.** Cerebral Organoids Generated with the STEMdiff™ Cerebral Organoid Kit Show Improved Quality, Yield and Marker Expression Than Those Generated with Unqualified Reagents

(A) Day 40 cerebral organoids generated using the STEMdiff™ Cerebral Organoid Kit develop thick tissue-like structures that are over 1 mm in diameter. (B) Day 40 organoids generated using unqualified reagents do not develop thick tissues and instead generate large fluid-filled cysts. (C) RT-qPCR analysis shows upregulation of both neural progenitor and mature neurons in cerebral organoids generated using the STEMdiff™ Cerebral Organoid Kit, (D) whereas organoids generated using unqualified reagents show poor neuronal marker expression (average ± SEM n = 2 per cell line, 6 organoids per analysis). Data were normalized to 18S/TBP and compared to undifferentiated hPSC control. Scale bar for (A) and (B) =1 mm.

## Cerebral Organoids Have Been Used to Model:

- Microcephaly[3]
- ZIKA Virus-Induced Microcephaly[4-7]
- Effect of Cocaine[8]
- Autism Spectrum Disorder/Timothy Syndrome[9]
- Alzheimer's Disease[10]
- Cancer[11]
- Miller-Dieker Syndrome[12-13]

## Product Information

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| STEMdiff™ Cerebral Organoid Kit | 1 Kit | 08570 |
| STEMdiff™ Cerebral Organoid Maturation Kit | 1 Kit | 08571 |

# References


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## STEMdiff™ Cerebral Organoid Kit

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