Application of REPRODUCIBLE HUMAN CORTICAL ORGANOIDS

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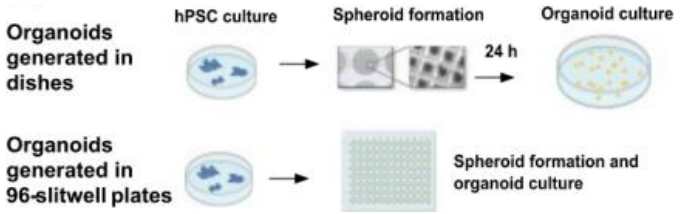
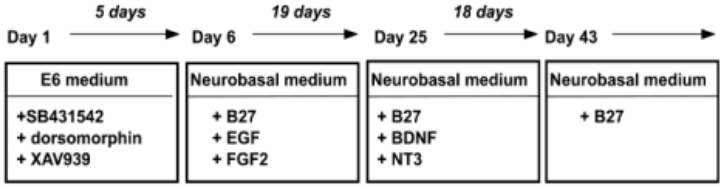
Human pluripotent stem cell (hPSC)-derived cortical organoids serve as advanced models to study human brain development and neurological disorders. These organoids recapitulate key developmental processes such as neurogenesis and gliogenesis, enabling long-term studies of cortical structure and function. However, existing protocols often suffer from low efficiency and variability across different hPSC lines, limiting reproducibility. This study presents an improved protocol using PrimeSurface™ 96 Slit-well Plate that increases production efficiency and consistency across multiple donor lines. This enhanced platform allows for sensitive detection of disease-related metabolic alterations, facilitating deeper insights into the mechanisms of neurodegenerative diseases.

Method

In this application note, cerebral cortical organoids were generated from hPSCs using 96 Slit-well Plate . Cells formed spheroids within the plate wells and were subsequently cultured with specific patterning factors. For the control group, spheroids were first formed in a microwell dish and then transferred to a standard dish 24 hours later for organoid culture. Throughout the culture period, the morphology and size of organoids were regularly monitored, and immunostaining was performed to assess neuronal marker expression. The use of the 96 Slit-well Plate allowed for efficient and consistent large-scale production of organoids.





Results

- Organoids cultured in the 96 Slit-well Plate exhibited minimal reduction in number, high survival rates, uniform size, and stable growth.

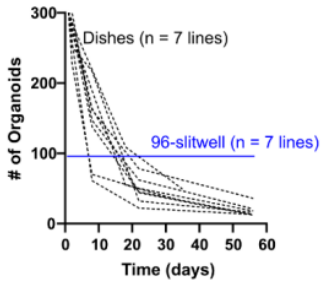


Fig. 1 The number of organoids over time showed less reduction in the 96-slitwell plate (solid blue line) compared to dish controls (dashed line) .

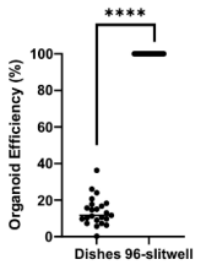


Fig. 2 Organoid production efficiency after 20 days of culture. High organoid production efficiency was maintained in the 96-slitwell plate.

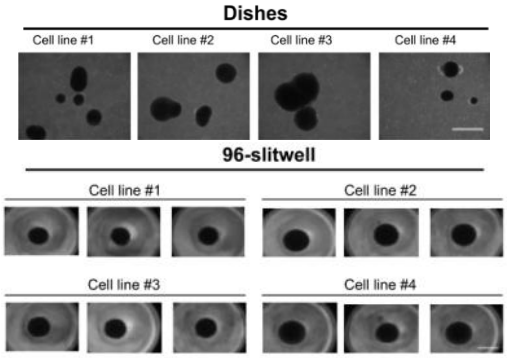


Fig. 3 Organoids generated in dish controls exhibited a range of sizes, while organoids in the 96 Slit-well Plate demonstrated stable growth.

- Spheroids generated in the 96 Slit-well Plate showed significantly higher expression of pluripotency-related genes and demonstrated uniform size distribution across multiple hPSC lines in comparison to the control group.

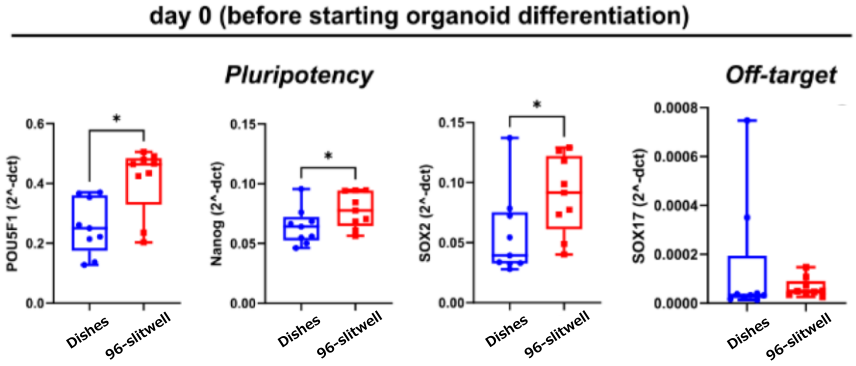
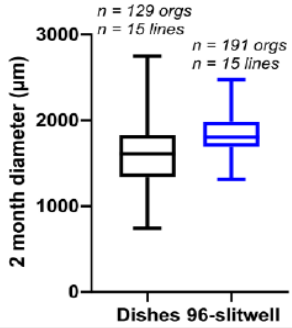


Fig. 4 Gene expression at the spheroid formation stage (day 0). The hPSC spheroids in the 96-slitwell plate showed significantly higher expression of pluripotency-associated genes POU5F1/OCT4, NANOG, and SOX2 compared to spheroids formed in dish controls.



	Dishes	96-slitwell
Coefficient of variation	25.50%	12.76%

Fig. 5 Diameter variation after culturing 15 hPSC lines for two months. Although organoids generated by both methods reached similar diameters, the reproducibility between organoids and between cell lines was significantly improved with the 96-slit well plate.

Discussion

Culturing with the 96 Slit-well Plate significantly improved organoid survival and size uniformity, leading to more stable growth than conventional methods. This reduced variability between cell lines and experiments, enhancing reproducibility and reliability. The consistent formation of uniform initial spheroids also played a crucial role in supporting culture consistency.

The 96 Slit-well Plate provides a uniform, stable culture environment that enables large-scale, efficient organoid production. It reduces experimental variability and contributes to the standardization of organoid-based disease models.

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