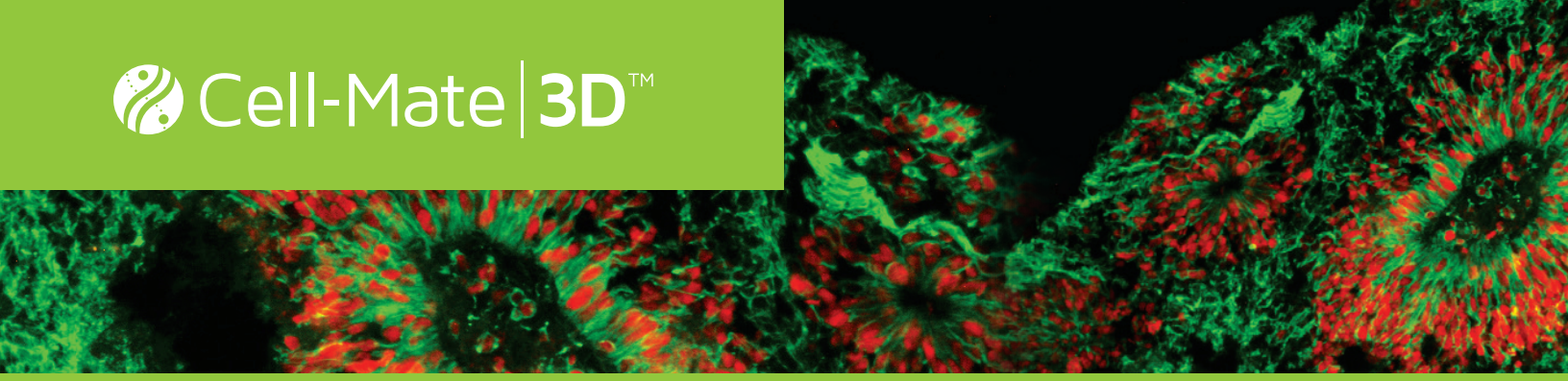
Cell-Mate 3D™



CEREBRAL ORGANOID generation

A Simpler, Easier Method for Cerebral Organoid Generation

Consider a simple and easy method for iPSC derived organoid generation, published by Lindborg et al. in Stem Cell Translational Medicine that uses only Cell-Mate3D™ matrix and maintenance media.[1] In this study, cerebral organoids are generated in which forebrain, hindbrain, and cortical markers are present in 4 week old cultures.[1]

Does your system generate cerebral organoids in 3 weeks? If not, consider Cell-Mate3D!

iPSCs are embedded into the matrix as whole colonies, cut into several pieces, and then cultured in maintenance media.[1] After 3 weeks, cerebral organoids bud out of the surfaces of the matrix and continue to grow for several weeks.[1]

Major advantages over other protocols include

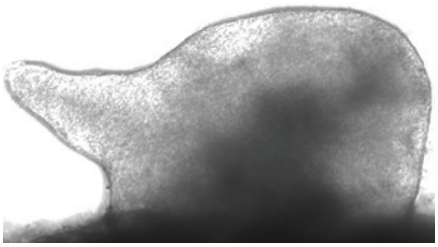
- Use of chemically defined reagents, increasing translatability to clinical applications
- Setup is fast and simple, saving time
- Requires no additional neural induction factors
- Overall cost is 30% lower compared to Lancaster method[2]

Researchers benefit because

- Neural Tube and Neural Rosette structures are present[1]
- Disease modeling of brain disorders can be achieved
- Cerebral Organoids can be utilized for pharmaceutical and toxicology screening[3]

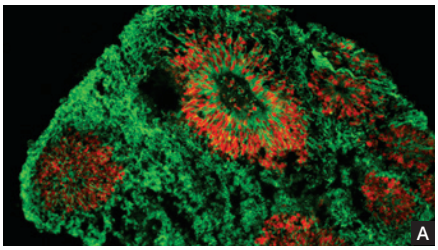
Consider These Results!

For detailed information, see the article "Rapid Induction of Cerebral Organoids From Human Induced Pluripotent Stem Cells Using a Chemically Defined Hydrogel and Defined Cell Culture Medium" published in Stem Cells Translational Medicine in July 2016.

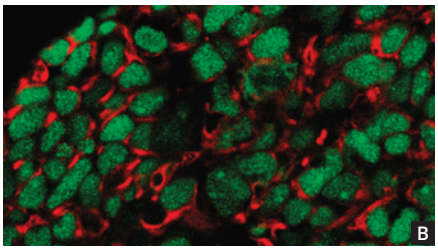


Organoid Formation
(4 weeks in culture)[4]

Fig 1. Emergence of Cerebral Organoid.
Using phase contrast microscopy, cerebral organoids 2mm in diameter are observed budding out of the matrix surface at 4 weeks.



Neural Tube-like Structures[a]
Sox1 (red) Nestin (green)[4]



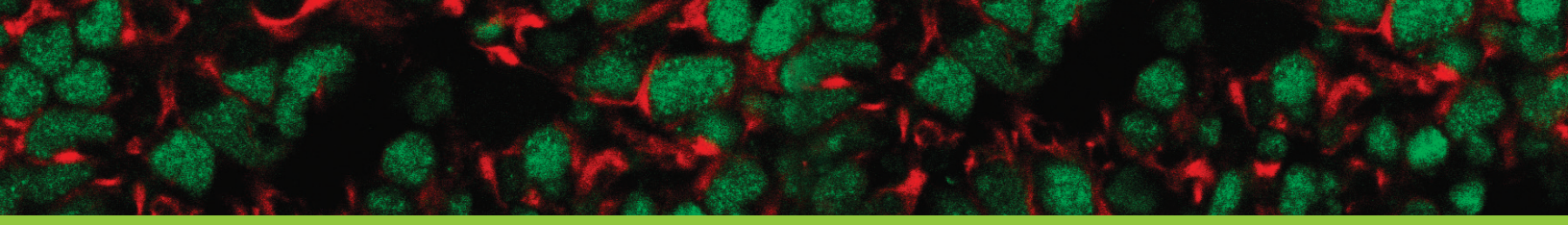
Neural Rosettes[b]
Sox2 (green) Nestin (red)[4]

Fig 2. Formation of Organized Structures.
Cerebral Organoids were cryosectioned and stained (a, b.) Sox1 (red)/Nestin (green) staining and organization suggests formation of neural tube-like structures (a). Sox2 (green)/Nestin (red) staining and organization suggests formation of neural rosettes (b).

What researchers are saying about using Cell-Mate3D™ to generate Cerebral Organoids...

"Cell-Mate3D is a great tool for making organoids without an EHS matrix."
– Scientist at Large Research Institution

BRTI™ Life Sciences



Protocol

Generation of Cerebral Organoids using Cell-Mate3D™

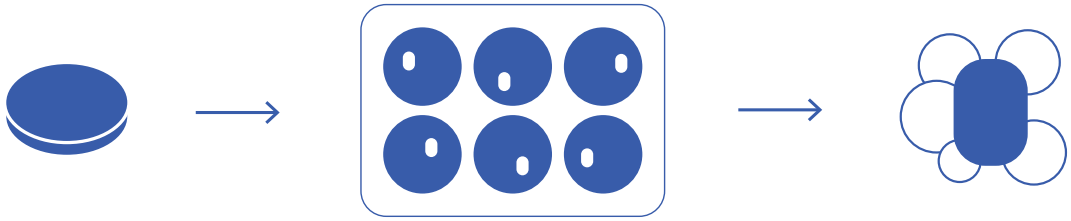
Required Reagents

- Cell-Mate3D™ matrix (BRTI Life Sciences product# CM-1001 or CM-1002)
- E8 culture media (Life Technologies product# A1517001)
- 6 well culture dish
- Sterile blade

Protocol

1. Refer to BRTI Life Sciences protocol for creating the Cell-Mate3D™ matrix
2. Harvest iPSCs from 2D culture maintaining the iPSCs as whole colonies
3. Following the Cell-Mate3D™ protocol, embed iPSC colonies into the Cell-Mate3D™ matrix
   - For a 500µL matrix, embed 19M cells
   - For a 250µL matrix, embed 9.5M cells
4. Prepare a 6 well culture plate by transferring E8 media into 5 wells. Use 5mL of media per well.
5. Cut the matrix into approximately 100µL sections
6. Organoids will begin to appear on the surface of the matrix after 3 weeks
7. Organoids can be analyzed by:
   - Histological methods
   - Cryosectioning and staining methods
   - Protein isolation and expression analysis
   - RNA isolation and expression analysis

Organoid Generation Workflow



- Expand iPSCs
- Embed in Cell-Mate3D™ Hydrogel
- Culture in Maintenance Media
- Organoids (white circles) Emerge from Hydrogel Surfaces (purple)

References

1.) Lindborg BA, Brekke JH, Vegoe AL, et al. Rapid Induction of Cerebral Organoids From Human Induced Pluripotent Stem Cells Using a Chemically Defined Hydrogel and Defined Cell Culture Medium. Stem Cells Transl Med. 2016 Jul;5(7):970-9. 2.) Cost of organoid generation using Lancaster method is approximately $150 per organoid and cost of O'Brien method is approximately $100 per organoid. Cost of organoid generation is reduced because neural induction factors are not needed. The Scientist. September 1, 2015. http://www.the-scientist.com/?articles.view/articleNo/43842/title/Orchestrating-Organoids/ accessed Feb 22 2017. 3.) Schwartz MP, Hou Z, Propson NE, et al. Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(40):12516-12521. doi:10.1073/pnas.1516645112. 4.) Images courtesy of Dr. Timothy O'Brien at the University of Minnesota.

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