

# Human iPSC-Derived Midbrain Organoids



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Catalog #200-0790  
#200-0791  
#200-0792  
#200-0793

48 Differentiated Organoids  
96 Differentiated Organoids  
48 Mature Organoids  
96 Mature Organoids

## Product Description

Human iPSC-Derived Midbrain Organoids are guided, three-dimensional in vitro models with a cellular composition and structural organization representative of the developing human midbrain. These organoids are manufactured from the human induced pluripotent stem cell (iPSC) line, Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using STEMdiff™ Midbrain Organoid Differentiation Kit (Catalog #100-1096). After a two-week recovery period, Human iPSC-Derived Midbrain Organoids are ready for assessment or use in downstream applications, and can be maintained for long-term culture using STEMdiff™ Neural Organoid Maintenance Medium (Catalog #100-0120). Differentiated and Mature iPSC-Derived Midbrain Organoids express markers for midbrain dopaminergic progenitors (FOXA2 and LMX1A) and midbrain neurons (TH, GIRK2, and PITX3).

Cells used to generate this product were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

## Stability and Storage

This product is shipped at ambient temperature. Keep the box upright when handling; do not invert. Transfer organoids to maintenance culture immediately upon receipt; do not freeze.

## Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, 7, and 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

## Materials Required but Not Included

PRODUCT NAME	CATALOG #
6-Well Ultra-Low Adherent Plates for Suspension Cultures*	100-0083
Serological pipettes, 5 mL	e.g. 38003
STEMdiff™ Neural Organoid Maintenance Kit	100-0120

\* If ultra-low attachment plates are not available, tissue culture-treated cultureware or non tissue culture-treated cultureware (e.g. Catalog #38015 or #38016) can be used if pre-treated with Anti-Adherence Rinsing Solution (Catalog #07010) to prevent cell attachment.

## Preparation of Reagents and Materials

### A. PREPARATION OF MEDIUM

Use sterile technique to prepare complete STEMdiff™ Neural Organoid Maintenance Medium (STEMdiff™ Neural Organoid Basal Medium 2 + STEMdiff™ Neural Organoid Supplement A). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw STEMdiff™ Neural Organoid Supplement A at room temperature (15 - 25°C). Mix thoroughly.

NOTE: Once thawed, use supplement immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.

- Add 2 mL of STEMdiff™ Neural Organoid Supplement A to 98 mL of STEMdiff™ Neural Organoid Basal Medium 2. Mix thoroughly. Warm to room temperature before use.

NOTE: STEMdiff™ Neural Organoid Basal Medium 2 is viscous; pipette slowly to ensure medium is transferred effectively.

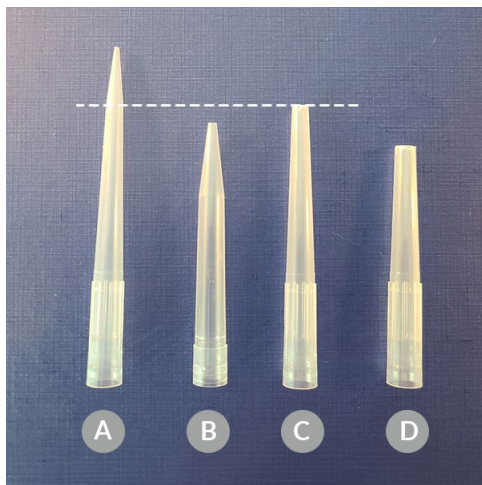
NOTE: If not used immediately, store complete STEMdiff™ Neural Organoid Maintenance Medium at 2 - 8°C for up to 3 weeks. Do not exceed the shelf life of the individual components.

## B. PREPARING CUT PIPETTE TIPS FOR ORGANOID TRANSFER

Wide-bore pipette tips are required to transfer differentiated and mature Human iPSC-Derived Midbrain Organoids out of the 96-well plate and into a maintenance culture plate. Standard 1 mL pipette tips are not suitable for handling these organoids.

In a biosafety cabinet using sterile scissors, cut the end off of a standard 1 mL (p1000) pipette tip to an appropriate bore size as follows:

- **Differentiated Organoids:** cut ~ 1.5 cm from the tip (typically at the bevel) to widen the bore size to ~3 mm (Figure 1C).
- **Mature Organoids:** cut ~2 cm from the tip to widen the bore size to ~4 - 5 mm (Figure 1D).



**Figure 1. Example of 1000 µL Pipette Tips Required to Transfer Organoids**

(A) Standard 1000 µL pipette tips (bore size of < 1 mm) and (B) commercially-available wide-bore 1000 µL pipette tips (bore size of ~1.5 mm) are not suitable for handling Differentiated or Mature Human iPSC-Derived Midbrain Organoids. (C) Standard 1000 µL pipette tips cut 1.5 cm from the end (bore size of ~3 mm) are suitable for handling Differentiated Organoids (Catalog #200-0790/200-0791). (D) Standard 1000 µL pipette tips cut 2 cm from the end (bore size of ~4 - 5 mm) are suitable for handling Mature Organoids (Catalog #200-0792/200-0793).

## Directions for Use

The following instructions are for transferring one 96-well plate containing 48 Human iPSC-Derived Midbrain Organoids to a 6-well Ultra-Low Adherent Plate for Suspension Cultures, and should be performed immediately upon receipt. Use sterile technique throughout the protocol. If using other cultureware for organoid maintenance, adjust volumes and plating densities accordingly. Allow the organoids to recover for at least two weeks before use in downstream applications.

**CRITICAL: Do not freeze.** Human iPSC-Derived Midbrain Organoids contain post-mitotic neurons and cell types that are not amenable to cryopreservation.

NOTE: If Ultra-Low Adherent Plates for Suspension Cultures are not available, 6-well tissue culture-treated plates pre-treated with Anti-Adherence Rinsing Solution (Catalog #07010) may also be used to prevent cell attachment. Coat the surface of each well with 1 mL of Anti-Adherence Rinsing Solution, then immediately remove and discard. Add 1 mL of sterile D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>; Catalog #37350) to each well and set the plate aside. Discard the D-PBS before use.

- Prepare a 6-Well Ultra-Low Adherent Plate for Suspension Cultures containing 3 mL/well of STEMdiff™ Neural Organoid Maintenance Medium (Preparation of Reagents and Materials section A) and set aside.
- Unpack the 96-well plate of Human iPSC-Derived Midbrain Organoids from the shipping box as follows:
  - Retrieve the inner box located between the insulation panels of the styrofoam container. Keep the inner box upright.
  - Carefully lift the double-bagged plate of iPSC-Derived Midbrain Organoids out the inner box while keeping the plate level. Remove the plate from both bags.
  - Remove the rubber bands from the plate while holding the lid in place. Do not open the lid or remove the adhesive membrane covering the wells.
- Centrifuge the plate at 100 x g for 3 minutes in a swinging-bucket centrifuge fitted with plate adaptors to force the organoids to settle to the bottom of the wells.

4. Wipe the outside of the plate with 70% ethanol or isopropanol and transfer to a biosafety cabinet.
5. Remove the plate lid and carefully peel back the adhesive membrane as follows:
  - a. Grab the white strip of paper on the side of the adhesive membrane with one hand while securing the plate in place with the other.
  - b. In a controlled and continuous motion, peel back the membrane by pulling towards the opposite side of the plate.
6. Using an appropriately-sized cut pipette tip (Preparation of Reagents and Materials section B), transfer up to 8 organoids per well of the 6-well plate prepared in step 1. Refer to Table 1 for recommended volumes and culture densities for other cultureware.

**Table 1. Recommended Volumes and Organoid Culture Density for Various Cultureware**

CULTUREWARE	VOLUME OF NEURAL ORGANOID MAINTENANCE MEDIUM PER WELL OR DISH (mL)	MAXIMUM NUMBER OF ORGANOID PER WELL OR DISH
6-well plate or 60 mm dish	3	8
12-well plate	1.5	4
24-well plate	1	2

7. Distribute the organoids evenly across the wells by moving the plate in several quick, short, back-and-forth and side-to-side motions. Visually inspect the plate to ensure minimal contact between organoids.
8. Carefully place the plate on a level surface in a 37°C and 5% CO<sub>2</sub> incubator.  
NOTE: Alternatively, place the plate on an orbital shaker (e.g. INFORS HT Celltron orbital shaker set at 68 rpm) in a 37°C and 5% CO<sub>2</sub> incubator.
9. Allow the organoids to recover for at least two weeks before use. Perform full-medium changes every 2 - 3 days as follows:
  - a. Warm a sufficient volume of STEMdiff™ Neural Organoid Maintenance Medium to room temperature (15 - 25°C).
  - b. Tilt the plate and allow the organoids to settle to the bottom of the wells (~15 - 30 seconds).
  - c. Slowly remove the medium using a 5 mL serological pipette at the slowest setting, leaving behind a small amount of medium (i.e. ~100 - 200 µL).
  - d. Add 3 mL/well of fresh STEMdiff™ Neural Organoid Maintenance Medium.
  - e. Incubate at 37°C and 5% CO<sub>2</sub> for 2 - 3 days.
10. Midbrain organoids are ready for assessment or use in downstream applications after two weeks of full-medium changes. For long-term culture, continue full-medium changes with STEMdiff™ Neural Organoid Maintenance Medium every 2 - 3 days.

## Assessment of Midbrain Organoids

Human iPSC-Derived Midbrain organoids may be assessed by immunostaining using the following antibodies:

- Anti-human tyrosine hydroxylase (TH) antibody (Pel-Freeze, P40101-150)
- Anti-human LMX1A antibody
- Anti-human HNF-3 beta/FoxA2 antibody (R&D Systems, AF2400)
- Anti-human GIRK2 antibody (Alomone labs, APC-006)

Markers specific to midbrain cell phenotypes can be detected in the peripheral areas of midbrain organoids. These markers include FOXA2+ and LMX1A+ midbrain progenitor cells, TH+ dopaminergic neurons, and GIRK2+ dopaminergic cells located in the substantia nigra in vivo.

## Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit [www.stemcell.com/hPSCNCworkflow](http://www.stemcell.com/hPSCNCworkflow), or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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