Miltenyi Biotec

StemMACS™ Cerebral OrganoidDiff Kit XF
human

Order no. 130-136-490

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1. Description

This product is for research use only.

Components

1 × 500 mL StemMACS Cerebral OrganoidDiff Basal Medium 1 XF, human

2 × 125 mL StemMACS Cerebral OrganoidDiff Basal Medium 2 XF, human

1 × 2 mL StemMACS Cerebral OrganoidDiff EB Formation Supplement XF (20×), human

1 × 2.4 mL StemMACS Cerebral OrganoidDiff Induction 1 Supplement XF (30×), human

1 × 2.4 mL StemMACS Cerebral OrganoidDiff Induction 2 Supplement XF (70×), human

1 × 6.6 mL StemMACS Cerebral OrganoidDiff Cultivation Supplement XF (50×), human

Specifications

Prepared EB Formation Medium
Osmolality: 290–370 mOsmol/kg
pH: 7.1–7.5

Prepared Neural Induction Medium I
Osmolality: 290–370 mOsmol/kg
pH: 7.1–7.5

Prepared Neural Induction Medium II
Osmolality: 300–380 mOsmol/kg
pH: 7.1–7.5

Prepared Cultivation Medium
Osmolality: 300–380 mOsmol/kg
pH: 7.1–7.5

Capacity

For 2 assays. One assay corresponds to one 96-well plate.

Storage

Upon arrival, store StemMACS Cerebral OrganoidDiff Basal Media XF, human protected from light at +2 to +8 °C. Store StemMACS Cerebral OrganoidDiff Kit XF Supplements protected from light at –20 °C. The expiration date is indicated on the label.

1.1 Background information

Pluripotent stem cell–derived brain organoids are one of the most frequently used organoid systems. They are mainly used for studying developmental pathways, modeling developmental diseases, and drug testing. StemMACS Cerebral OrganoidDiff Kit XF, human is a complete, ready-to-use, and xeno-free cell culture system for the efficient differentiation of human pluripotent stem cells (hPSCs) into cerebral organoids. The kit is composed of four media to promote the matrix-free generation of PSC-derived brain organoids in 30 days of culture. The StemMACS Cerebral OrganoidDiff Kit XF, human allows the generation of up to 192 individual, cerebral organoids in two 96-well plates. On day 15, organoids are transferred to 10 cm dishes with up to 48 organoids per dish.

1.2 Applications

- Differentiation and cultivation of cerebral organoids from hPSCs.

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1.3 Reagent and instrument requirements

For hPSC expansion:

- StemMACS iPS-Brew XF, human (# 130-104-368) or StemMACS PSC-Brew XF, human (# 130-127-865)
- 6-well plates

For hPSC differentiation into cerebral organoids:

- Induced pluripotent stem cells (iPSCs) maintained, e.g., in StemMACS iPS-Brew XF, human or StemMACS PSC-Brew XF, human
- TrypLE™ Select Enzyme (1×) (# 12563029, Thermo Fisher)
- Soybean Trypsin Inhibitor (0.5 mg/mL)
- Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}
- 2 × 96-well ultra low attachment (ULA) round bottom plates
- 4 × 10 cm dishes (non-treated or ULA)
- 3D shaker

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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2. Protocol

2.1 Protocol overview

Day	Action	Plate	Complete medium*
0	Plate cells	96-well ULA round bottom plate	Embryoid Body (EB) Formation Medium
1	Change media		Neural Induction Medium I
3	Change media		Neural Induction Medium I
5	Change media		Neural Induction Medium II
7–13	Change media every second day		Neural Induction Medium II
15	Transfer to dish, change media	10 cm dish on 3D shaker	Cultivation Medium
17–29	Change media every 2–3 days		Cultivation Medium

* For media preparation refer to table 2.

Table 1: Protocol overview.

2.2 Preparation of complete media

▲ Kit components should not be substituted or mixed with those from other kits or lots.

▲ Media volumes below are for one assay using one assay starting with one 96-well plate.

▲ All steps should be performed under sterile conditions.

▲ Prepare complete media according to table 2 on the day of first use. If only part of a supplement is used, aliquot and store at –20 °C. Avoid freeze-thaw cycles.

▲ Equilibrate complete media to room temperature (+20 to +25 °C) prior to use.

Complete media	Component	Amount (mL)
Embryoid Body (EB) Formation Medium (day 0)	StemMACS Cerebral OrganoidDiff Basal Medium 1 XF, human	19
	StemMACS Cerebral OrganoidDiff EB Formation Supplement XF (20×), human	1
Neural Induction Medium I (day 1)	StemMACS Cerebral OrganoidDiff Basal Medium 1 XF, human	34.8
	StemMACS Cerebral OrganoidDiff Induction 1 Supplement XF (30×), human	1.2
Neural Induction Medium II (day 5)	StemMACS Cerebral OrganoidDiff Basal Medium 2 XF, human	83.8
	StemMACS Cerebral OrganoidDiff Induction 2 Supplement XF (70×), human	1.2
Cultivation Medium (day 15)	StemMACS Cerebral OrganoidDiff Basal Medium 1 XF, human	161.7
	StemMACS Cerebral OrganoidDiff Cultivation Supplement XF (50×), human	3.3

Table 2: Preparation of complete media.

1. Thaw supplements at +2 to +8 °C overnight. Thawed material must be used immediately.

   ▲ **Note:** If a white precipitate occurs, pipette up and down to dissolve it.

   ▲ **Note:** Neural Induction Medium II contains a viscosity enhancer. Due to higher viscosity compared to standard medium, slow pipetting is recommended.

2. Shake the supplements vials vigorously.
3. Prepare complete media according to table 2.

   ▲ **Note:** (Optional) Antibiotic solutions might be added to prevent contamination, e.g. 100 U/mL penicillin and 100 µg/ mL streptomycin.

2.3 Detailed differentiation protocol

Day 0
Harvesting of hPSCs and seeding of organoids

▲ Prepare EB Formation Medium as indicated in table 2. The confluency of the hPSC culture should not exceed 85%.

1. Aspirate StemMACS iPS Brew-XF, human from iPSCs and wash each well of a 6-well plate with 2 mL of DPBS without Ca^{2+} and Mg^{2+}.
2. Add 1 mL/well TrypLE Select Enzyme (1×).
3. Incubate for 5 minutes at +37 °C.
4. Stop enzymatic reaction by adding 1 mL/well of Soybean Trypsin Inhibitor (0.5 mg/mL).
5. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
6. Transfer cell suspension into a suitable tube. Determine the cell number and viability. Viability should be >90%.
7. Gently mix cells again to obtain a homogeneous cell suspension.
8. Transfer the desired cell number into a new tube. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.

   ▲ **Note:** For initial experiments, a cell number titration might be required. A range between 6,000 and 15,000 cells/well is recommended.

9. Resuspend the cells in a sufficient amount of EB Formation Medium. Per well 150 µL of cells in medium are needed.
10. Transfer cells into a 96-well ULA round bottom plate with 150 µL cells in medium/well.

    ▲ **Note:** When working with reservoirs and multichannel pipettes, calculate an overfill of at least 15 wells.

11. Centrifuge plate at 300×g for 5 minutes. Place plate into an incubator (+37 °C, 5% CO_2). Leave plate undisturbed for 24 hours.

Day 1
Replacing medium with Neural Induction Medium I

▲ Prepare Neural Induction Medium I as indicated in table 2.

▲ Organoids should show a round shape.

1. One day (24 hours) after seeding, carefully aspirate as much EB Formation Medium as possible from the culture plate without aspirating organoids.
2. Replace with 150 µL/well of Neural Induction Medium I.

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Day 3
Replacing medium with Neural Induction Medium I

▲ Organoids should show a round shape. They should be compact and show transparent edges.

1. Two days (48 hours) after previous feeding, aspirate as much Neural Induction Medium I as possible from the culture plate, but maximum of 120 µL to avoid organoid loss.
2. Replace with 150 µL/well of Neural Induction Medium I.

Day 5
Replacing medium with Neural Induction Medium II

▲ Prepare Neural Induction Medium II as indicated in table 2.

▲ Due to higher viscosity compared to standard medium, slow pipetting is recommended.

1. Two days (48 hours) after previous feeding, aspirate Neural Induction Medium I from the culture plate.

   ▲ **Note:** Carefully aspirate as much medium as possible without aspirating organoids.
2. Replace with 150 µL/well of Neural Induction Medium II.

Day 7–13
Exchanging medium with Neural Induction Medium II every second day

▲ Over time organoids should increase in size and start to form buds, thereby losing their round shape.

1. Replace old Neural Induction Medium II with 150 µL/well fresh Neural Induction Medium II every 48 hours.

   ▲ **Note:** Carefully aspirate as much medium as possible without aspirating organoids.

Day 15
Transfering organoids to a 10 cm dish

▲ Prepare Cultivation Medium as indicated in table 2.

1. Transfer the organoids into a 10 cm dish using a wide bore pipette tip.
2. Tilt the dish to let organoids sink. Aspirate as much medium as possible without aspirating organoids.
3. Add 10 mL of Cultivation Medium.
4. Place the dish on a 3D shaker (47 rpm) in an incubator (+37 °C, 5% CO_2).

   ▲ **Note:** The optimal rpm value might depend on the used shaker model.

Day 17–29
Exchanging medium with Cultivation Medium every 2–3 days

1. Tilt the dish to let organoids sink. Aspirate as much medium as possible without aspirating organoids.
2. Add 10 mL of Cultivation Medium.
3. Place the dish back on a 3D shaker (47 rpm) in an incubator (+37 °C, 5% CO_2).

   ▲ **Note:** The optimal rpm value might depend on the used shaker model.

Day 30
Analysis of organoids or continued cultivation with the StemMACS Cerebral Organoid Cultivation Medium XF, human

Organoids can now be analyzed by flow cytometry, immune fluorescence, single-cell sequencing, etc.

Refer to application protocol "**hPSC-derived cerebral organoids for human brain development research**" for more information about different downstream applications.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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