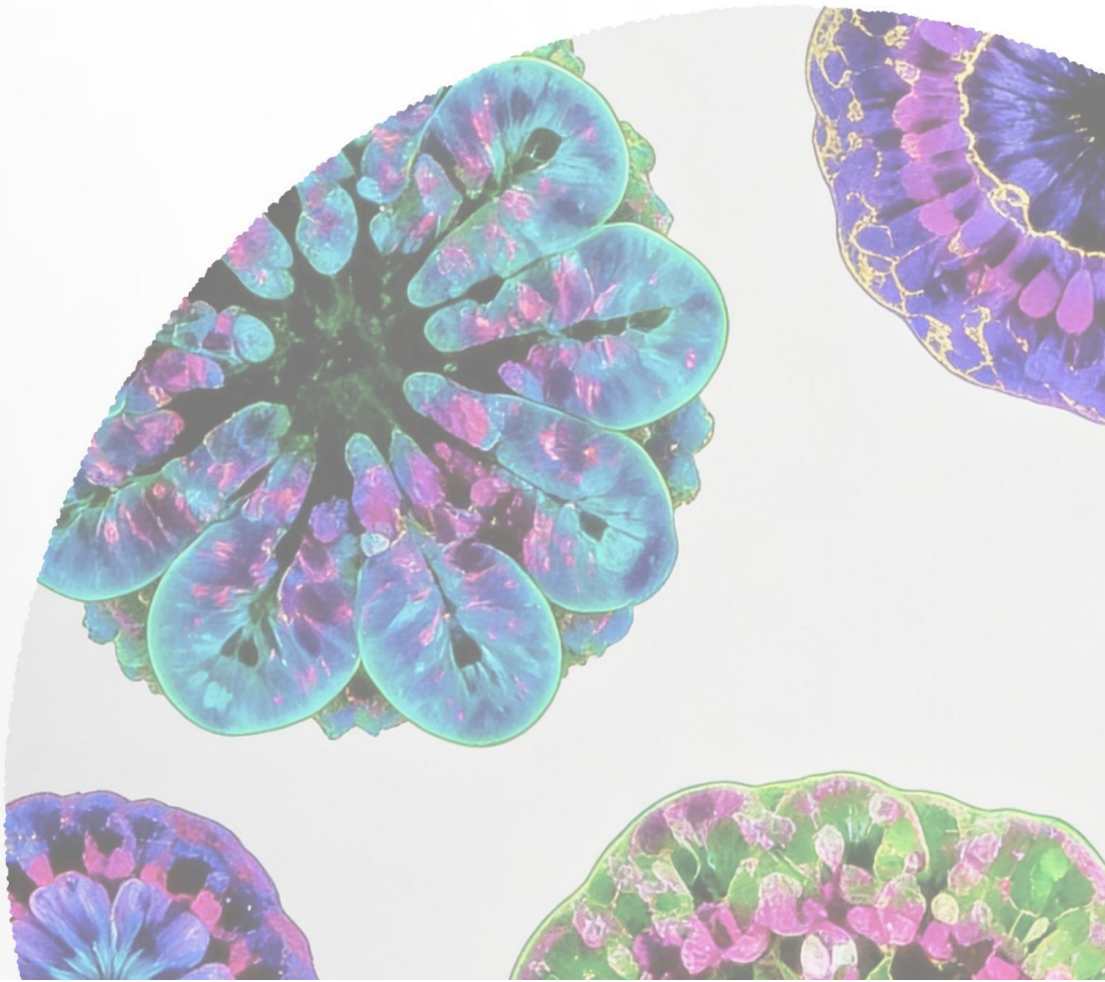
ACRO BIOSYSTEMS | Organoids

hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

RIPO-BWM01-60 mL | 120 | 360 mL

Reimagining human and disease models on a dish



hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

Cat. No. RIPO-BWM01-60 mL

Product Description

Human iPSC-Derived Dorsal Forebrain Organoid Maintenance Medium (Cat. No. RIPO-BWM01) is a serum-free medium designed for the culture and maintenance of Human iPSC-Derived Dorsal Forebrain Organoids (Cat. No. CIPO-BWL01/CIPO-BWL02). The medium supports organoid viability, morphology, and maintenance of dorsal forebrain lineage characteristics during long-term culture. It is formulated to help preserve organoid structure and neural marker expression after recovery and throughout the recommended culture period. This medium is manufactured under controlled conditions to support batch-to-batch consistency and is free of antibiotics.

Product Information

All components listed below are provided as part of the Dorsal Forebrain Organoid Maintenance Kit (Cat. No. RIPO-BWM01) and are not sold separately.

Table 1 shows the supplied volumes, including extra volume for handling loss. For medium preparation, please follow the working volumes specified in Table 2.

Antibiotics are not included in this kit and must be purchased separately for complete maintenance medium preparation. Recommended Penicillin-Streptomycin (P/S) or Antibiotic-Antimycotic solution are listed in the Materials section.

Cat. No.	Component	Component Cat. No.	Size	Storage	Shelf Life
RIPO-BWM01	Neural Organoid Basal Medium	RIPO-BWM01-C01	58 mL	2-8℃	Stable until the expiry date stated on the label
	Neural Organoid Supplement 1	RIPO-BWM01-C02	0.6 mL	2-8℃	Stable until the expiry date stated on the label
RIPO-BWM01-1	Neural Organoid Supplement 2	RIPO-BWM01-1-C01	1.2 mL total; 0.4 mL × 3 vials	-80℃	Stable until the expiry date stated on the label
	Neural Organoid Supplement 3	RIPO-BWM01-1-C02	0.3 mL total; 0.1 mL × 3 vials	-80℃	Stable until the expiry date stated on the label

Table 1. The product information of hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

Materials (Not include in our kit)

Product Name	Cat. No.	Manufacturer
6-well ULA plate	CP-21	ACROBiosystems
24-well ULA plate	WP24-5CCUSH	ALPHA PLUS
48-well ULA plate	WP48-5CCUSH	ALPHA PLUS
96-well ULA plate	CP-22	ACROBiosystems
3mL disposable Pasteur pipette, extended type, sterilized	PP102030	Jet Biofil
hiPSC-Derived Dorsal Forebrain Organoid	CIPO-BWL01	ACROBiosystems
Sterile serum bottle/centrifuge tube	N/A	N/A
Antibiotic-Antimycotic	15240062	Gibco
Penicillin-Streptomycin (P/S)	15140122	Gibco

Equipment

Equipment	Specification / Notes
CO_2 Incubator	37°C, 5% CO_2
Orbital Shaker	20 mm shaking diameter
Water Bath	37°C
Biosafety Cabinet	Aseptic handling
Freezer	-80°C
Refrigerator	4°C

Experimental Procedure

1. Medium Preparation

1.1 Upon receipt of the hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit, verify all components against Table 1 and store them immediately under the recommended conditions. If any component is missing, damaged or leaking, please document the issue with photos and contact sales support immediately.

1.2 Use sterile technique to prepare complete hiPSC-Derived Dorsal Forebrain Organoid Maintenance Medium following the instruction below. This kit is intended for use only with hiPSC-derived Dorsal Forebrain Organoids (CIPO-BWL01/CIPO-BWL02).

1.3 Before use, take one vial of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 from the -80°C freezer. Thaw the supplements overnight at 2-8°C or at room temperature for 1 h. Use only the supplements are completely thawed. Do not aliquot or refreeze Neural Organoid Supplement 2 and Supplement 3 after thawing, as repeated handling or freeze-thaw cycles can compromise its stability and performance.

1.4 Prepare the complete maintenance medium in a sterile serum bottle or centrifuge tube. Gently mix all components at room temperature (18-25°C) according to the suggested volumes listed in Table 2. Avoid excessive bubble formation during mixing. ***Note:*** *Prepare an appropriate volume of complete maintenance medium according to experimental needs. For each preparation, use one vial each of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 and mix them with the corresponding volume of basal medium and other required components. Once mixed, store the complete maintenance medium at 2-8°C and use within 2 weeks.*

1.5 For each medium change, transfer only the required volume of complete maintenance medium into a sterile centrifuge tube and pre-warm in 37°C water bath for 15 min before use. Calculate the required volume based on the number of organoids and the culture plate format and number of wells used. Avoid repeatedly warming the entire bottle of complete maintenance medium, as repeated temperature changes may affect medium stability. Keep the remaining complete maintenance medium at 2-8°C.

Medium	Component	Suggested Volume per Preparation (1 Vial Set)	Storage and Handling
hiPSC-Derived Dorsal Forebrain Organoid Maintenance medium	Neural Organoid Basal medium	15.3 mL	Store at 2-8°C before use.
	Neural Organoid Supplement 1	0.16 mL	Store at 2-8°C before use.
	Neural Organoid Supplement 2	0.32 mL	Thaw one vial before each preparation. Do not refreeze after thawing.
	Neural Organoid Supplement 3	0.024 mL	Thaw one vial before each preparation. Do not refreeze after thawing.
	Antibiotics (100X)	0.16 mL	Add during medium preparation.
	Complete Maintenance Medium	~16 mL	Store at 2-8°C and use within 2 weeks.
	Each preparation uses one vial each of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 to prepare approximately 16 mL of complete maintenance medium. The kit supports three preparations in total.		

Table 2. Preparation of hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

2. Package Inspection

2.1 Upon receipt, recover Ready-to-use Human iPSC-Derived Dorsal Forebrain Organoids as soon as possible. Before opening, inspect the outer package, cryovial, transport medium, and organoid integrity.

2.2 Proceed only if the cryovial is intact, the medium is clear with no signs of freezing or precipitation, and the organoid remains visible, spherical, and intact (Figure 1). If damage, leakage, turbidity, freezing, precipitation, or organoid disintegration is observed, do not open the cryovial. Document the issue with photos and contact sales support immediately.



*Figure 1. Example of Dorsal Forebrain Organoid Packaging. * The dorsal forebrain organoid is expected to appear intact upon receipt.*

3. Organoid Recovery

3.1 Pre-warm only the required volume of complete Dorsal Forebrain Organoid Maintenance Medium at 37°C for 15 min before use. Calculate the volume based on the number of wells to be used. Avoid repeated warming of the medium bottle, as frequent temperature changes may affect medium stability. Keep the remaining complete medium at 2-8°C. For example, when preparing medium for one well of a 6-well plate, transfer 5 mL of medium into a sterile 15 mL centrifuge tube and pre-warm the aliquot at 37°C for 15 min before medium change.

Culture Format	Maintenance Medium Volume	Organoids per Well	Medium Change Frequency	Orbital Shaker
6-well ULA plate	5 mL	16	**Option 1:** Three medium changes per week: perform two half-medium changes on Day 1 and Day 3, followed by one full-medium change on Day 5. **Option 2:** Perform a full-medium change every 3 days.	Yes, 100 rpm
24-well ULA plate	1 mL	1		Optional, static or 60 rpm orbital shaker
48-well ULA plate	500 µL	1		Optional, static or 60 rpm orbital shaker
96-well ULA plate	200 µL	1	Daily	No, static culture

Table 1. Recommended Medium Volumes and Culture Conditions for Dorsal Forebrain Organoids

3.2 Open the 2 mL cryovial and gently transfer the organoids into the wells of an ULA plate using a Pasteur pipette.

3.3 Remove the residual transport medium from each well containing the organoids. Add pre-warmed maintenance medium to each well, ensuring that the organoids are fully submerged and not exposed to air. ***Note:*** *Medium change should be performed gently by adding pre-warmed maintenance medium slowly along the well wall to minimize shear stress and avoid mechanical damage.*

3.4 Culture the organoids at 37°C with 5% CO_2 under shaking conditions for 3 days to allow recovery. Perform full-medium changes every 2 days without touching or damaging the organoids. A 6-well ULA plate (100 rpm) is recommended for the recovery phase.

4. Organoid Maintenance

4.1 After the 3-day recovery phase, the organoids are ready for downstream assays. Before proceeding, confirm the organoid morphology and medium condition. Organoids should maintain a near-spherical morphology with clear, smooth edges and no obvious surrounding cell debris.

4.2 For continued culture, maintain the organoids at 37°C with 5% CO_2 under either static or orbital shaking conditions, following the culture conditions and medium-change schedule described in Table 1. Monitor organoid morphology and culture status regularly.

4.3. Protocols for Ca^{2+} imaging, immunostaining, qPCR and MEA recording are available online.



Figure 2. Orbital shaker culture of dorsal forebrain organoids at 37°C with 5% CO_2.

Related Product

Product	Cat. No.
hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit	RIPO-BWM01
6-well ULA plate	CP-21
96-well ULA plate	CP-22

hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit
Cat. No. RIPO-BWM01-120 mL

Product Description

Human iPSC-Derived Dorsal Forebrain Organoid Maintenance Medium (Cat. No. RIPO-BWM01) is a serum-free medium designed for the culture and maintenance of Human iPSC-Derived Dorsal Forebrain Organoids (Cat. No. CIPO-BWL01/CIPO-BWL02). The medium supports organoid viability, morphology, and maintenance of dorsal forebrain lineage characteristics during long-term culture. It is formulated to help preserve organoid structure and neural marker expression after recovery and throughout the recommended culture period. This medium is manufactured under controlled conditions to support batch-to-batch consistency and is free of antibiotics.

Product Information

All components listed below are provided as part of the Dorsal Forebrain Organoid Maintenance Kit (Cat. No. RIPO-BWM01) and are not sold separately.

Table 1 shows the supplied volumes, including extra volume for handling loss. For medium preparation, please follow the working volumes specified in Table 2.

Antibiotics are not included in this kit and must be purchased separately for complete maintenance medium preparation. Recommended Penicillin-Streptomycin (P/S) or Antibiotic-Antimycotic solution are listed in the Materials section.

Cat. No.	Component	Component Cat. No.	Size	Storage	Shelf Life
RIPO-BWM01	Neural Organoid Basal Medium	RIPO-BWM01-C01	116 mL	2-8℃	Stable until the expiry date stated on the label
	Neural Organoid Supplement 1	RIPO-BWM01-C02	1.2 mL	2-8℃	Stable until the expiry date stated on the label
RIPO-BWM01-1	Neural Organoid Supplement 2	RIPO-BWM01-1-C01	2.4 mL total; 0.8 mL × 3 vials	-80℃	Stable until the expiry date stated on the label
	Neural Organoid Supplement 3	RIPO-BWM01-1-C02	0.3 mL total; 0.1 mL × 3 vials	-80℃	Stable until the expiry date stated on the label

Table 1. The product information of hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

Materials (Not include in our kit)

Product Name	Cat. No.	Manufacturer
6-well ULA plate	CP-21	ACROBiosystems
24-well ULA plate	WP24-5CCUSH	ALPHA PLUS
48-well ULA plate	WP48-5CCUSH	ALPHA PLUS
96-well ULA plate	CP-22	ACROBiosystems
3mL disposable Pasteur pipette, extended type, sterilized	PP102030	Jet Biofil
hiPSC-Derived Dorsal Forebrain Organoid	CIPO-BWL01	ACROBiosystems
Sterile serum bottle/centrifuge tube	N/A	N/A
Antibiotic-Antimycotic	15240062	Gibco
Penicillin-Streptomycin (P/S)	15140122	Gibco

Equipment

Equipment	Specification / Notes
CO_2 Incubator	37°C, 5% CO_2
Orbital Shaker	20 mm shaking diameter
Water Bath	37°C
Biosafety Cabinet	Aseptic handling
Freezer	-80°C
Refrigerator	4°C

Experimental Procedure

1. Medium Preparation

1.1 Upon receipt of the hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit, verify all components against Table 1 and store them immediately under the recommended conditions. If any component is missing, damaged or leaking, please document the issue with photos and contact sales support immediately.

1.2 Use sterile technique to prepare complete hiPSC-Derived Dorsal Forebrain Organoid Maintenance Medium following the instruction below. This kit is intended for use only with hiPSC-derived Dorsal Forebrain Organoids (CIPO-BWL01/CIPO-BWL02).

1.3 Before use, take one vial of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 from the -80°C freezer. Thaw the supplements overnight at 2-8°C or at room temperature for 1 h. Use only the supplements are completely thawed. Do not aliquot or refreeze Neural Organoid Supplement 2 and Supplement 3 after thawing, as repeated handling or freeze-thaw cycles can compromise its stability and performance.

1.4 Prepare the complete maintenance medium in a sterile serum bottle or centrifuge tube. Gently mix all components at room temperature (18-25°C) according to the suggested volumes listed in Table 2. Avoid excessive bubble formation during mixing. ***Note:*** *Prepare an appropriate volume of complete maintenance medium according to experimental needs. For each preparation, use one vial each of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 and mix them with the corresponding volume of basal medium and other required components. Once mixed, store the complete maintenance medium at 2-8°C and use within 2 weeks.*

1.5 For each medium change, transfer only the required volume of complete maintenance medium into a sterile centrifuge tube and pre-warm in 37°C water bath for 15 min before use. Calculate the required volume based on the number of organoids and the culture plate format and number of wells used. Avoid repeatedly warming the entire bottle of complete maintenance medium, as repeated temperature changes may affect medium stability. Keep the remaining complete maintenance medium at 2-8°C.

Medium	Component	Suggested Volume per Preparation (1 Vial Set)	Storage and Handling
hiPSC-Derived Dorsal Forebrain Organoid Maintenance medium	Neural Organoid Basal medium	31.6 mL	Store at 2-8°C before use.
	Neural Organoid Supplement 1	0.33 mL	Store at 2-8°C before use.
	Neural Organoid Supplement 2	0.66 mL	Thaw one vial before each preparation. Do not refreeze after thawing.
	Neural Organoid Supplement 3	0.05 mL	Thaw one vial before each preparation. Do not refreeze after thawing.
	Antibiotics (100X)	0.33 mL	Add during medium preparation.
	Complete Maintenance Medium	~33 mL	Store at 2-8°C and use within 2 weeks.
	Each preparation uses one vial each of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 to prepare approximately 33 mL of complete maintenance medium. The kit supports three preparations in total.		

Table 2. Preparation of hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

2. Package Inspection

2.1 Upon receipt, recover Ready-to-use Human iPSC-Derived Dorsal Forebrain Organoids as soon as possible. Before opening, inspect the outer package, cryovial, transport medium, and organoid integrity.

2.2 Proceed only if the cryovial is intact, the medium is clear with no signs of freezing or precipitation, and the organoid remains visible, spherical, and intact (Figure 1). If damage, leakage, turbidity, freezing, precipitation, or organoid disintegration is observed, do not open the cryovial. Document the issue with photos and contact sales support immediately.



*Figure 1. Example of Dorsal Forebrain Organoid Packaging. * The dorsal forebrain organoid is expected to appear intact upon receipt.*

3. Organoid Recovery

3.1 Pre-warm only the required volume of complete Dorsal Forebrain Organoid Maintenance Medium at 37°C for 15 min before use. Calculate the volume based on the number of wells to be used. Avoid repeated warming of the medium bottle, as frequent temperature changes may affect medium stability. Keep the remaining complete medium at 2-8°C. For example, when preparing medium for one well of a 6-well plate, transfer 5 mL of medium into a sterile 15 mL centrifuge tube and pre-warm the aliquot at 37°C for 15 min before medium change.

Culture Format	Maintenance Medium Volume	Organoids per Well	Medium Change Frequency	Orbital Shaker
6-well ULA plate	5 mL	16	**Option 1:** Three medium changes per week: perform two half-medium changes on Day 1 and Day 3, followed by one full-medium change on Day 5. **Option 2:** Perform a full-medium change every 3 days.	Yes, 100 rpm
24-well ULA plate	1 mL	1		Optional, static or 60 rpm orbital shaker
48-well ULA plate	500 µL	1		Optional, static or 60 rpm orbital shaker
96-well ULA plate	200 µL	1	Daily	No, static culture

Table 1. Recommended Medium Volumes and Culture Conditions for Dorsal Forebrain Organoids

3.2 Open the 2 mL cryovial and gently transfer the organoids into the wells of an ULA plate using a Pasteur pipette.

3.3 Remove the residual transport medium from each well containing the organoids. Add pre-warmed maintenance medium to each well, ensuring that the organoids are fully submerged and not exposed to air. ***Note:*** *Medium change should be performed gently by adding pre-warmed maintenance medium slowly along the well wall to minimize shear stress and avoid mechanical damage.*

3.4 Culture the organoids at 37°C with 5% CO_2 under shaking conditions for 3 days to allow recovery. Perform full-medium changes every 2 days without touching or damaging the organoids. A 6-well ULA plate (100 rpm) is recommended for the recovery phase.

4. Organoid Maintenance

4.1 After the 3-day recovery phase, the organoids are ready for downstream assays. Before proceeding, confirm the organoid morphology and medium condition. Organoids should maintain a near-spherical morphology with clear, smooth edges and no obvious surrounding cell debris.

4.2 For continued culture, maintain the organoids at 37°C with 5% CO_2 under either static or orbital shaking conditions, following the culture conditions and medium-change schedule described in Table 1. Monitor organoid morphology and culture status regularly.

4.3. Protocols for Ca^{2+} imaging, immunostaining, qPCR and MEA recording are available online.



Figure 2. Orbital shaker culture of dorsal forebrain organoids at 37°C with 5% CO_2.

Related Product

Product	Cat. No.
hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit	RIPO-BWM01
6-well ULA plate	CP-21
96-well ULA plate	CP-22

hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit
Cat. No. RIPO-BWM01-360 mL

Product Description

Human iPSC-Derived Dorsal Forebrain Organoid Maintenance Medium (Cat. No. RIPO-BWM01) is a serum-free medium designed for the culture and maintenance of Human iPSC-Derived Dorsal Forebrain Organoids (Cat. No. CIPO-BWL01/CIPO-BWL02). The medium supports organoid viability, morphology, and maintenance of dorsal forebrain lineage characteristics during long-term culture. It is formulated to help preserve organoid structure and neural marker expression after recovery and throughout the recommended culture period. This medium is manufactured under controlled conditions to support batch-to-batch consistency and is free of antibiotics.

Product Information

All components listed below are provided as part of the Dorsal Forebrain Organoid Maintenance Kit (Cat. No. RIPO-BWM01) and are not sold separately.

Table 1 shows the supplied volumes, including extra volume for handling loss. For medium preparation, please follow the working volumes specified in Table 2.

Antibiotics are not included in this kit and must be purchased separately for complete maintenance medium preparation. Recommended Penicillin-Streptomycin (P/S) or Antibiotic-Antimycotic solution are listed in the Materials section.

Cat. No.	Component	Component Cat. No.	Size	Storage	Shelf Life
RIPO-BWM01	Neural Organoid Basal Medium	RIPO-BWM01-C01	348 mL	2-8℃	Stable until the expiry date stated on the label
	Neural Organoid Supplement 1	RIPO-BWM01-C02	3.6 mL	2-8℃	Stable until the expiry date stated on the label
RIPO-BWM01-1	Neural Organoid Supplement 2	RIPO-BWM01-1-C01	7.2 mL total; 2.4 mL × 3 vials	-80℃	Stable until the expiry date stated on the label
	Neural Organoid Supplement 3	RIPO-BWM01-1-C02	0.6 mL total; 0.2 mL × 3 vials	-80℃	Stable until the expiry date stated on the label

Table 1. The product information of hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

Materials (Not include in our kit)

Product Name	Cat. No.	Manufacturer
6-well ULA plate	CP-21	ACROBiosystems
24-well ULA plate	WP24-5CCUSH	ALPHA PLUS
48-well ULA plate	WP48-5CCUSH	ALPHA PLUS
96-well ULA plate	CP-22	ACROBiosystems
3mL disposable Pasteur pipette, extended type, sterilized	PP102030	Jet Biofil
hiPSC-Derived Dorsal Forebrain Organoid	CIPO-BWL01	ACROBiosystems
Sterile serum bottle/centrifuge tube	N/A	N/A
Antibiotic-Antimycotic	15240062	Gibco
Penicillin-Streptomycin (P/S)	15140122	Gibco

Equipment

Equipment	Specification / Notes
CO_2 Incubator	37°C, 5% CO_2
Orbital Shaker	20 mm shaking diameter
Water Bath	37°C
Biosafety Cabinet	Aseptic handling
Freezer	-80°C
Refrigerator	4°C

Experimental Procedure

1. Medium Preparation

1.1 Upon receipt of the hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit, verify all components against Table 1 and store them immediately under the recommended conditions. If any component is missing, damaged or leaking, please document the issue with photos and contact sales support immediately.

1.2 Use sterile technique to prepare complete hiPSC-Derived Dorsal Forebrain Organoid Maintenance Medium following the instruction below. This kit is intended for use only with hiPSC-derived Dorsal Forebrain Organoids (CIPO-BWL01/CIPO-BWL02).

1.3 Before use, take one vial of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 from the -80°C freezer. Thaw the supplements overnight at 2-8°C or at room temperature for 1 h. Use only the supplements are completely thawed. Do not aliquot or refreeze Neural Organoid Supplement 2 and Supplement 3 after thawing, as repeated handling or freeze-thaw cycles can compromise its stability and performance.

1.4 Prepare the complete maintenance medium in a sterile serum bottle or centrifuge tube. Gently mix all components at room temperature (18-25°C) according to the suggested volumes listed in Table 2. Avoid excessive bubble formation during mixing. ***Note:*** *Prepare an appropriate volume of complete maintenance medium according to experimental needs. For each preparation, use one vial each of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 and mix them with the corresponding volume of basal medium and other required components. Once mixed, store the complete maintenance medium at 2-8°C and use within 2 weeks.*

1.5 For each medium change, transfer only the required volume of complete maintenance medium into a sterile centrifuge tube and pre-warm in 37°C water bath for 15 min before use. Calculate the required volume based on the number of organoids and the culture plate format and number of wells used. Avoid repeatedly warming the entire bottle of complete maintenance medium, as repeated temperature changes may affect medium stability. Keep the remaining complete maintenance medium at 2-8°C.

Medium	Component	Suggested Volume per Preparation (1 Vial Set)	Storage and Handling
hiPSC-Derived Dorsal Forebrain Organoid Maintenance medium	Neural Organoid Basal medium	95.9 mL	Store at 2-8°C before use.
	Neural Organoid Supplement 1	1 mL	Store at 2-8°C before use.
	Neural Organoid Supplement 2	2 mL	Thaw one vial before each preparation. Do not refreeze after thawing.
	Neural Organoid Supplement 3	0.15 mL	Thaw one vial before each preparation. Do not refreeze after thawing.
	Antibiotics (100X)	1 mL	Add during medium preparation.
	Complete Maintenance Medium	~100 mL	Store at 2-8°C and use within 2 weeks.
	Each preparation uses one vial each of Neural Organoid Supplement 2 and Neural Organoid Supplement 3 to prepare approximately 100 mL of complete maintenance medium. The kit supports three preparations in total.		

Table 2. Preparation of hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit

2. Package Inspection

2.1 Upon receipt, recover Ready-to-use Human iPSC-Derived Dorsal Forebrain Organoids as soon as possible. Before opening, inspect the outer package, cryovial, transport medium, and organoid integrity.

2.2 Proceed only if the cryovial is intact, the medium is clear with no signs of freezing or precipitation, and the organoid remains visible, spherical, and intact (Figure 1). If damage, leakage, turbidity, freezing, precipitation, or organoid disintegration is observed, do not open the cryovial. Document the issue with photos and contact sales support immediately.



*Figure 1. Example of Dorsal Forebrain Organoid Packaging. * The dorsal forebrain organoid is expected to appear intact upon receipt.*

3. Organoid Recovery

3.1 Pre-warm only the required volume of complete Dorsal Forebrain Organoid Maintenance Medium at 37°C for 15 min before use. Calculate the volume based on the number of wells to be used. Avoid repeated warming of the medium bottle, as frequent temperature changes may affect medium stability. Keep the remaining complete medium at 2-8°C. For example, when preparing medium for one well of a 6-well plate, transfer 5 mL of medium into a sterile 15 mL centrifuge tube and pre-warm the aliquot at 37°C for 15 min before medium change.

Culture Format	Maintenance Medium Volume	Organoids per Well	Medium Change Frequency	Orbital Shaker
6-well ULA plate	5 mL	16	**Option 1:** Three medium changes per week: perform two half-medium changes on Day 1 and Day 3, followed by one full-medium change on Day 5. **Option 2:** Perform a full-medium change every 3 days.	Yes, 100 rpm
24-well ULA plate	1 mL	1		Optional, static or 60 rpm orbital shaker
48-well ULA plate	500 µL	1		Optional, static or 60 rpm orbital shaker
96-well ULA plate	200 µL	1	Daily	No, static culture

Table 1. Recommended Medium Volumes and Culture Conditions for Dorsal Forebrain Organoids

3.2 Open the 2 mL cryovial and gently transfer the organoids into the wells of an ULA plate using a Pasteur pipette.

3.3 Remove the residual transport medium from each well containing the organoids. Add pre-warmed maintenance medium to each well, ensuring that the organoids are fully submerged and not exposed to air. ***Note:*** *Medium change should be performed gently by adding pre-warmed maintenance medium slowly along the well wall to minimize shear stress and avoid mechanical damage.*

3.4 Culture the organoids at 37°C with 5% CO_2 under shaking conditions for 3 days to allow recovery. Perform full-medium changes every 2 days without touching or damaging the organoids. A 6-well ULA plate (100 rpm) is recommended for the recovery phase.

4. Organoid Maintenance

4.1 After the 3-day recovery phase, the organoids are ready for downstream assays. Before proceeding, confirm the organoid morphology and medium condition. Organoids should maintain a near-spherical morphology with clear, smooth edges and no obvious surrounding cell debris.

4.2 For continued culture, maintain the organoids at 37°C with 5% CO_2 under either static or orbital shaking conditions, following the culture conditions and medium-change schedule described in Table 1. Monitor organoid morphology and culture status regularly.

4.3. Protocols for Ca^{2+} imaging, immunostaining, qPCR and MEA recording are available online.



Figure 2. Orbital shaker culture of dorsal forebrain organoids at 37°C with 5% CO_2.

Related Product

Product	Cat. No.
hiPSC-Derived Dorsal Forebrain Organoid Maintenance Kit	RIPO-BWM01
6-well ULA plate	CP-21
96-well ULA plate	CP-22