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ab317486 ioMotor Neurons – Human iPSC-Derived ioMotor Neurons

View ab317486 ioMotor Neurons - Human iPSC-Derived motor neurons datasheet:

http://www.abcam.com/ab317486

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For conversion into consistent, mature, functional motor neurons providing a high quality human model for the study of neurological activity and disease.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ioMotor Neurons rapidly mature into functional microglia after revival in the recommended medium. They are delivered in a convenient cryopreserved format and provide a homogeneous and reproducible model for motor neuron cells.

ioMotor Neurons form a homogeneous population of cells, displaying classical neuronal morphology and neurite outgrowth, within 4 days post revival. Cells express pan-neuronal markers MAP2 and TUBB3, cholinergic markers ChAT and VAChT and motor neuron-specific markers MNX1(HB9) and ISL1/2, as assessed by both ICC and RT-qPCR.

Bulk RNA sequencing of ioMotor Neurons demonstrates a rapid acquisition of a motor neuron signature. The expression of the key motor neuron marker genes MNX1, FOXP1, and ISL2 is detected in the culture from as early as 1 day post-thaw. On day 11, close to 80% of cells express MNX1 by single cell RNAseq. ioMotor Neurons show spontaneous neuronal activity with increasing firing rate over 40 days in culture, as shown by multielectrode array activity (MEA).

The protocol for the generation of ioMotor Neurons is a three-phase process. Phase 0 - Induction is carried out before distribution (Fig 1).

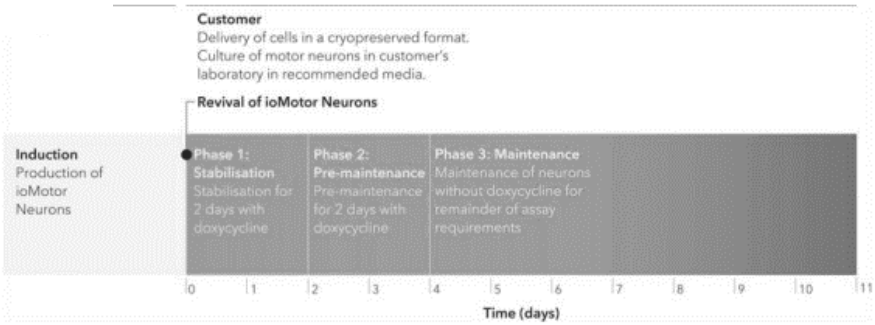


Figure 1 Schematic representation of the three-phase protocol to generate and culture ioMotor Neurons.

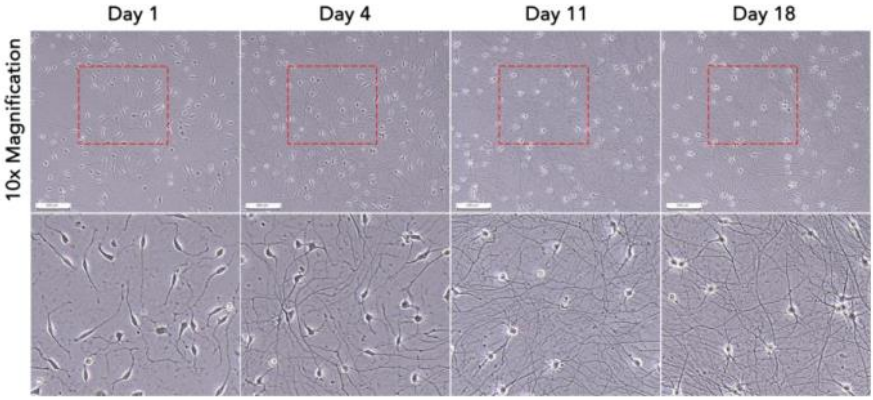
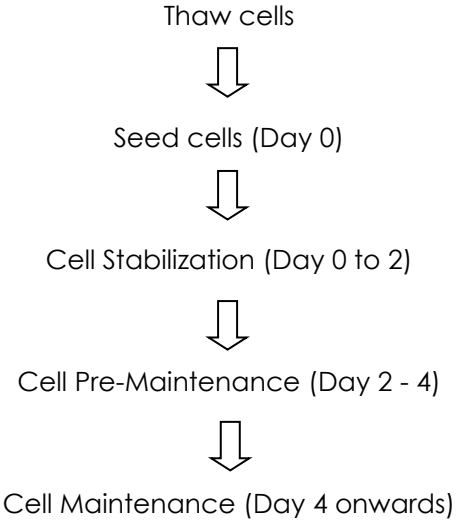


Figure 2 Photos of ioMotor Neurons after revival over the course of an 18 day culture period (Day 1 to 18 post-thawing; 10X magnification; scale bar: 200µm).

Manufacturer	**Phase 0 — Induction (day -3 to 0):** Human iPSCs are exposed to a 3-day induction protocol. This leads to irreversible loss of pluripotency, and the synchronised homogenous production of sensory neurons. The ioMotor Neurons are subsequently cryopreserved for distribution.
User	**Phase1 — Stabilization (day 0 to 2):** The ioMotor Neurons are revived at the user's laboratory using the recommended medium supplemented with doxycycline (96h) for sustained induction.
	Phase 2 — Pre-Maintenance (day 2 to 4): The ioMotor Neurons require a full media change with the recommended medium with doxycycline.
	Phase 3 — Maintenance (day 4 - onwards): The ioMotor Neurons require full media change with the recommended medium without doxycycline. Half media changes are subsequently performed every 48 hours onwards. Depending on assay requirements, ioMotor Neurons can be used over different lengths of time in the maintenance medium. Note: ioMotor Neurons have been maintained up to 42 days in the above conditions without impairment to function and culture attachment.

Table 1 Description of the three-phase protocol for the production and culture of ioMotor Neurons.

2. Protocol Summary



3. Materials Supplied and Storage

Transfer the vials of ioMotor Neurons to liquid nitrogen or to -150°C immediately after receipt.

The recommended reagents for the revival and maintenance of ioMotor Neurons can be found in Section 7.

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully culture the cells:

- Biological Safety Cabinet
- Normoxic cell culture incubator (37°C, 5% CO_2)
- 37°C water bath or equivalent
- Haemocytometer or automatic cell counter
- Liquid Nitrogen Storage Unit
- Standard tissue culture wares (pipettes, tips, culture plates)
- Bench Top Centrifuge
- Poly-D-Lysine hydrobromide
- Geltrex (LDEV Free, reduced GF) basement membrane matrix
- Sterile water
- DPBS (no calcium, no magnesium)
- DMEM/F-12
- B27 (50X)
- 20x Borate Buffer
- BDNF
- GDNF
- Neurobasal Medium
- NT-3
- FGF-2 (bFGF) 145 aa protein
- Retinoic Acid
- CHIR99021
- N-2 (100X)
- GlutaMAX (100X)
- 2-Mercaptoethanol (50mM)
- BSA fraction V (2.5%)
- Doxycycline (ab141091)

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the cells, please see the ioMotor Neurons datasheet on our website.

6. Reagent Preparation

6.1 Preparation of stock solutions

Reagent	Stock Solution	Working concentration
BDNF	10 µg/mL (500X solution) To prepare, reconstitute 10 ug in 1 mL of PBS containing 0.1% BSA	20ng/mL 2µL of stock solution per 1mL of medium
GDNF	10 µg /mL (1000x solution) To prepare, reconstitute 50 ug in 5 mL of PBS containing 0.1% BSA	10ng/mL 1µL of stock solution per 1mL of medium
NT3	50µg/mL (5000x solution) To prepare, reconstitute 25µg in 500µL PBS containing 0.1% BSA	10ng/mL 0.2µL of stock solution per 1mL of medium
CHIR99021	3mM (1000x solution) To prepare, reconstitute 10mg in 7.16mL of DMSO.	3µM 1µL of stock solution per 1mL of medium
FGF-2 (bFGF) 145 aa	4µg/mL (200x solution) To prepare, reconstitute 50µg solution in 12.5mL in PBS containing 0.1% BSA	20ng/mL 5µL of stock solution per 1mL of medium
Retinoic Acid	10mM (10,000X solution)	1µM

	To prepare, reconstitute 50mg in 16.64mL of DMSO	0.1µL of stock solution per 1mL of medium
Doxycycline (DOX) (ab141091)	2 mg/mL (2,000X solution) To prepare, reconstitute 20 mg in 10 mL of H_2O	1 µg/mL 0.5 µL of stock solution per 1 mL of medium

6.2 Preparation of ioMotor Neuron Medium

b:MN: Basal Motor Neuron Medium

Reagent/Media	For 200 mL	For 500 mL
DMEM/F-12	97.4 mL	243.55 mL
Neurobasal	97.4 mL	243.5 mL
GlutaMAX (100X)	2 mL	5 mL
N-2 (100X)	1 mL	2.5 mL
B-27 (50X)	2 mL	5 mL
BSA fraction V (7.5%)	0.2 mL	0.5 mL
2-Mercaptoethanol (final conc. 0.4 µM)	1.6 µL	4 µL

Δ Note: The basal medium is stable for 3 weeks at 4°C

stab:MN+D: Stabilisation Motor Neuron Medium

Reagent/Media	For 50 mL	For 200 mL
b:MN	49.7 mL	198.78 mL
CHIR 99021 (final conc. 3µM)	50 µL	200 µL
bFGF (final conc. 20µg/mL)	250 µL	1 mL
Retinoic Acid (final conc.1µM)	5 µL	20 µL

Doxycycline (final conc. 1µg/mL)	25 µL	100 µL

comp:MN: Complete Motor Neuron Medium

Reagent/Media	For 10 mL	For 50 mL
b:MN	9.97 mL	49.8 mL
BDNF (final conc. 20ng/mL)	20 µL	100 µL
GDNF (final conc. 10ng/mL)	10 µL	50 µL
NT3 (final conc. 10ng/mL)	2 µL	10 µL

Δ Note: The complete medium is better prepared fresh before each feeding.

comp:MN+D: Complete Motor Neuron Medium with Doxycycline

Reagent/Media	For 10 mL	For 50 mL
comp:MN	10 mL	50 mL
Doxycycline	5 µL	25 µL

Δ Note: The complete medium is better prepared fresh before each feeding.

6.3 Preparation of the PDL-Geltrex coating solution and coated vessels

For the preparation of PDL-Geltrex coated plates, treat culture vessels first with PDL solution and subsequently with Geltrex according to the protocol below:

6.3.1 Preparation of PDL coating solution:

6.3.1.1 Make up 50 mL of 1x borate buffer, by diluting 2.5 mL of the 20x stock with 47.5 mL of sterile water.

6.3.1.2 Resuspend a 5 mg vial of PDL in 50 mL of the 1x borate buffer, for a working concentration of 100 µg/mL. PDL

coating solution can be kept at 20°C for long-term storage.

6.3.2 PDL coating:

6.3.2.1 Calculate the total surface area to be coated.

6.3.2.2 Coat the surface area of your culture vessel with the PDL coating solution. Recommended coating volumes are shown in the table below

Coating solution	96 well	48 well	24 well	12 well	6 well
Poly-D-Lysine	42 µL	125 µL	250 µL	500 µL	1.25 mL

6.3.2.3 Incubate the coated plates, overnight at 37°C or for at least 3 hours at 37°C.

6.3.2.4 Aspirate PDL solution and then wash 3 times with sterile water. For each wash, use the same volume used for coating.

6.3.2.5 Asiprate the water and allow coated surfaces to dry completely in a laminar flow hood (without lids). This typically requires 60 minutes.

6.3.2.6 Proceed with Geltrex coating as described below. For the preparation of Geltrex aliquots and PDL-Geltrex coated plates, please follow the manufacturer's instructions. In brief:

6.4 Preparation of Geltrex aliquots

6.4.1 Remove Geltrex stock aliquots from -80°C and thaw on ices in a 4°C fridge overnight.

6.4.2 The next day, prepare aliquots according to foreseen use in order to minimize further freeze-thawing; store at -80°C.

Δ Note: Geltrex solidifies quickly at temperatures above 4°C — keep Geltrex on ice at all times. Depending on the volume, the smaller aliquots should take about 30 minutes to thaw on ice.

6.5 Geltrex coating

6.5.1 Calculate the total surface area to be coated.

6.5.2 Dilute the Geltrex 1:100 in chilled DMEM/F-12 (e.g. 100 µL in 10 mL)

6.5.3 Coat the surface area of your culture vessel with the Geltrex:DMEM/F-12 coating solution. We recommend the follow coating volumes:

Coating solution	96 well	48 well	24 well	12 well	6 well
Geltrex:DMEM	42 µL	125 µL	250 µL	500 µL	1.25 mL

6.5.4 Incubate the coated plates at 37°C for a minimum of 60 minutes. At the time of use, we recommend keeping the plates at room temperature for one hour before aspirating. Carefully aspirate off the excess Geltrex, then immediately plate the cells.

Δ Note: For long-term storage of Geltrex-coated plates, maintain the excess Geltrex in each well and seal the plate with parafilm. Geltrex coated plates can be kept at 4°C for 1 month.

7. Assay Procedure

7.1 Culture of Motor Neurons:

7.1.1 **Before starting**:

- Each ioMotor Neuron vial contains either ≥1 x10^6 viable cells. A seeding density of 30,000 cells/cm^2 is recommended. Prepare enough tissue culture vessels with PDL-Geltrex coating prior to thawing the cryovial(s) (see Section 6.3).
- Warm-up the water bath to 37°C.
- Allow basal motor neuron medium (b:MN) to reach room temperature prior to thawing the cells
- Prepare the stabilisation motor neuron medium supplemented with 1ug/mL doxycycline (D) (stab:MN+D) and heat to 37°C for revival (see Section 6.2).

7.1.2 **Cell Thawing**:

7.1.2.1 Remove the cryovial(s) from dry ice and immediately immerse into a 37°C waterbath (or similar) while maintaining a constant gentle agitation.

7.1.2.2 Remove the cryovial(s) from the water bath when only a very small ice cube is left visible (this should take approximately 2 minutes).

7.1.2.3 Spray the cryovial(s) with 70% ethanol and take it to a biological safety cabinet.

7.1.2.4 Transfer the cells from each vial into a 50 mL tube.

Δ Note: Freezing medium contains DMSO: minimize the time between thawing and centrifugation of cells.

7.1.2.5 Carefully wash each cryovial with 1mL of b:MN medium and add to the 50mL tube in a dropwise manner, agitating the tube occasionally to ensure the cell suspension is mixed with b:MN medium before centrifugation

7.1.2.6 Add a further 8 mL of b:MN medium per tube in a dropwise manner.

7.1.2.7 Centrifuge the cells at 350 x *g* for 5 minutes at room temperature.

7.1.2.8 Carefully remove the supernatant by aspiration.

7.1.2.9 Add 1 mL Add 1mL of stab:MN+D medium, per vial of cells thawed, to the cell pellet and gently resuspend the cells by pipetting up-and-down.

7.1.2.10 Count the cells including a cell viability marker. The typical recovery from one cryovial is ≥1x10^6 viable cells.

7.1.3 **Cell Seeding (Day 0)**:

7.1.3.1 Dilute the cell suspension to the required cell concentration using stab:MN+D medium to achieve the required seeding density for your desired experimental conditions. A seeding density of 30,000 cells/cm^2 is routinely used.

Plate format	Surface (cm^2)	mL/well	Cells/well
6 well	9.5	2.5	285,000
12 well	3.8	1.0	114,000
24 well	1.9	0.5	57,000
48 well	0.95	0.25	28,500
96 well	0.32	0.1	9,600

7.1.3.2 Aspirate the Geltrex coating solution from the culture vessel(s).

7.1.3.3 Directly add the required volume of cell suspension to the culture vessel(s).

7.1.3.4 Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO_2.

7.1.3.5 To ensure an even cell distribution, gently cross-shake the plate once on the incubator shelf (back and forth, side to side, 2-3 times).

7.1.4 **Cell Stabilization (Day 0 to 2):**

7.1.4.1 Day 1: 24 hours post thawing, gently aspirate the spent medium and replace it with fresh pre-warmed stab:MN+D medium.

Δ Note: Culture of ioMotor Neurons should be carried out with special care as neuronal cells are prone to mechanical stress which may cause detachment. It is recommended that for all medium replacements, medium aspiration and addition should be performed slowly and on the side of the well, using micropipettes instead of serological pipettes.

7.1.5 **Cell Pre-Maintenance (day 1 onwards):**

7.1.5.1 Day 2: 48 hours post thawing, completely replace the spent culture medium with fresh pre-warmed comp:MN+D medium.

7.1.5.2 Day 3: 72 hours post thawing, completely replace the spent culture medium with fresh pre-warmed comp:MN+D medium.

7.1.6 **Cell Maintenance (day 4 onwards):**

7.1.6.1 Day 4: Completely replace the culture medium in each well with the recommended volume of fresh pre-warmed comp:MN medium (no doxycycline).

7.1.6.2 Day 6 onwards: a half-media change every 48 hours, replacing 50% of the medium with fresh comp:MN medium (no doxycycline) is recommended.

8. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)