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ab317489

ioSensory Neurons – Human iPSC-Derived Sensory Neurons

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For conversion into consistent, mature, functional sensory 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

ioSensory Neurons rapidly mature into functional sensory neurons after revival in the recommended medium. They are delivered in a convenient cryopreserved format and provide a homogeneous and reproducible model for human sensory neurons.

ioSensory Neuron cultures consist mainly of a highly pure sensory neuron population (>99%) characterized by the expression of pan-sensory neuron markers, PRPH, BRN3A, ISL1, TUBB3. ioSensory Neurons have a nociceptor identity as shown by the expression of the key nociceptor markers, NTRK1 and TRPV1. These cells display spontaneous activity and show a functional nociceptor phenotype, as demonstrated by responsiveness to selective agonists for a number of thermosensitive TRP channels, including TRPV1, TRPM3, and TRPM8.

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

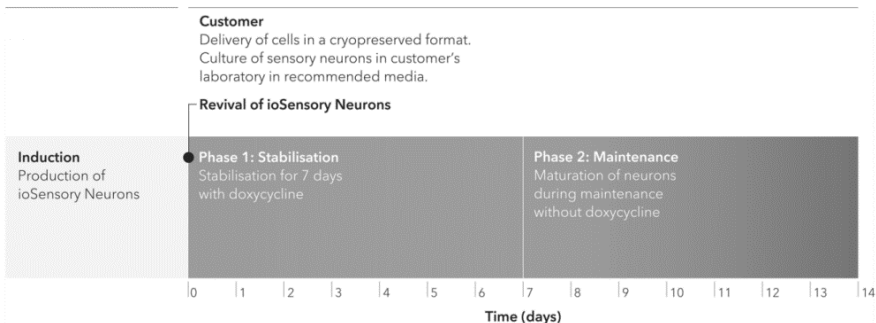


Figure 1 Schematic representation of the two-phase protocol to generate and culture ioSensory Neurons.

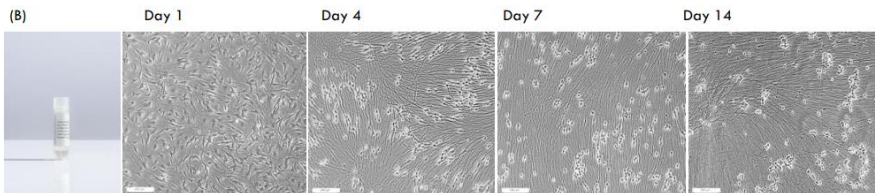
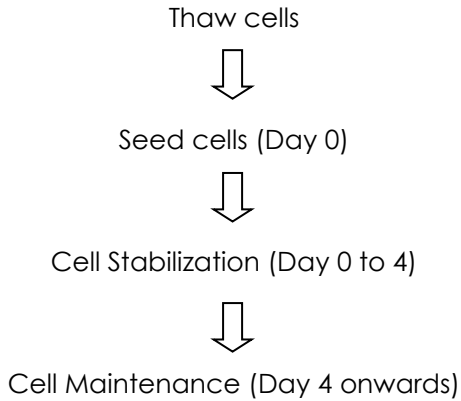


Figure 2 Photos of sensory neurons after revival over the course of the first 14 days of culture (Day 1 to 14 post-thawing; 10X magnification; scale bar: 200µm).

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 ioSensory Neurons are subsequently cryopreserved for distribution.
User	Phase 1 — Stabilization (day 0 to 7): The ioSensory Neurons are revived at the user's laboratory using the recommended medium supplemented with doxycycline (96h) for sustained induction.
	Phase 2 — Maintenance (day 7 onwards): Depending on assay requirements, the ioSensory Neurons can be used over different lengths of time in the maintenance medium. Note: ioSensory Neurons have been maintained up to 21 days in the above conditions without impairment to neuronal health, function and culture attachment.

Table 1 Description of the two-phase protocol for the production and culture of ioSensory Neurons.

2. Protocol Summary



3. Materials Supplied and Storage

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

Before the revival of ioSensory Neurons, prepare the tissue culture plates or flasks coated with PDL-Geltrex (refer to Section 6.3)

The recommended reagents for the revival and maintenance of ioSensory Neurons can be found in Section 4.

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% CO₂)
- 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
- Geltrex (Reduced GF)
- PDL-hydrobromide
- Borate buffer (20x)
- Sterile water
- DMEM/F-12
- Neurobasal medium
- N-2
- B27
- Glutamax
- 2-Mercaptoethanol
- Beta-NGF
- BDNF
- CNTF
- FGF-2 (bFGF) 145 aa protein
- Doxycycline (ab141091)
- Bovine Serum Albumin
- Dibutyl-cAMP

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 ioSensory Neurons cells datasheet on our website.

6. Reagent Preparation

6.1 Preparation of stock solutions

Reagent	Stock Solution	Working concentration
Beta-NGF	100 µg/mL (2,000X solution) To prepare, reconstitute 100 µg in 1000 µL of PBS containing 0.1% BSA	50 ng/mL 0.5 µL of stock solution per 1 mL of medium
BDNF	10 µg /mL (1,000X solution) To prepare, reconstitute 50 µg in 5000 µL of PBS containing 0.1% BSA	10 ng/mL 1 µL of stock solution per 1 mL of medium
CNTF	50 µg/mL (5,000X solution) To prepare, reconstitute 50 µg in 1000 µL of PBS containing 0.1% BSA	10 ng/mL 0.2 µL of stock solution per 1 mL of medium
Doxycycline (DOX) (ab141091)	2 mg/mL (2,000X solution) To prepare, reconstitute 20 mg in 10 mL of H ₂ O	1 µg/mL 0.5 µL of stock solution per 1 mL of medium
Dibutyl-cAMP	100 mM (1,000X solution) To prepare, reconstitute 100 mg in 2.04 mL of DMSO	0.1 mM 0.1 µL of stock solution per 1 mL of medium

6.2 Preparation of Sensory Neuron Medium

b:SN: Basal Sensory Neuron Medium

Reagent/Media	For 200 mL	For 500 mL
Neurobasal	192 mL	480 mL
Glutamax (100x)	2 mL	5 mL
N-2 (100x)	2 mL	5 mL
B27 (50x)	4 mL	10 mL

Δ Note: The basal medium is stable for 2 weeks at 4°C; Pen/Strep antibiotics can be added if required.

comp:SN: Complete Sensory Neuron Medium

Reagent/Media	For 50 mL	For 200 mL
b:SN	49.6 mL	198.5 mL
NGF (final conc. 50 ng/mL)	25 µL	100 µL
BDNF (final conc. 10 ng/mL)	50 µL	200 µL
CNTF (final conc. 10 ng/mL)	10 µL	40 µL
FGF (final conc. 20 ng/mL)	250 µL	1 mL
cAMP (final conc. 0.1 mM)	50 µL	200 µL

comp:SN+D: Doxycycline Supplemented Complete Sensory Neuron Medium

Reagent/Media	For 10 mL	For 50 mL
comp:SN	10 mL	50 mL
Doxycycline (final conc. 1 µg/mL)	5 µL	25 µL

Δ Note: The supplemented complete medium is better prepared fresh before each feeding. It is not recommended to use the supplemented complete medium for more than 4 days after preparation while stored at 4°C.

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.
- 6.3.1.3 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	64 µL	200 µL	400 µL	800 µL	2 mL

- 6.3.2.3 Incubate the coated plates, overnight at 37°C or for at least 3h 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 Aspirate the water and allow coated surfaces to dry completely in a laminar flow hood (without lids). This typically requires 30-60 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:

6.3.3 Preparation of Geltrex aliquots:

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

6.3.3.2 Depending on the volume, the smaller aliquots should take about 30 minutes to thaw on ice.

6.3.4 Geltrex coating

6.3.4.1 Calculate the total surface area to be coated.

6.3.4.2 Dilute the Geltrex 1:100 in chilled DMEM/F12 (e.g. 100 µL in 10 mL)

6.3.4.3 Coat the surface area of your culture vessel with the Geltrex:DMEM/F12 coating solution.

6.3.4.4 We recommend the follow coating volumes

Coating solution	96 well	48 well	24 well	12 well	6 well
Geltrex:DMEM	50 µL	125 µL	250 µL	500 µL	1 mL

6.3.4.5 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 Sensory Neurons:

7.1.1 Before starting:

- Each ioSensory Neurons vial contains either $\geq 2 \times 10^6$ viable cells. A seeding density of 60,000 cells/cm² is recommended. Prepare enough tissue culture vessels with Geltrex coating prior to reviving the cryovial(s) (see Section 6.3).
- Warm-up the water bath to 37°C.
- Warm-up basal sensory neuron medium (b:SN) medium to 37°C (see Section 6.2).
- Prepare the complete sensory neuron (comp:SN) medium supplemented by 1 µg/mL Doxycycline (comp:SN+D) 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 15 mL tube containing 1 mL of b:SN medium.
Δ Note: Freezing medium contains DMSO: minimize the time between thawing and centrifugation of cells.
- 7.1.2.5 Add a further 8 mL of b:SN medium per tube in a dropwise manner.
- 7.1.2.6 Carefully wash the cryovial(s) with 1 mL of b:SN medium and add it to the tube(s).
- 7.1.2.7 Centrifuge the cells at 300 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 of comp:SN+D medium to the cell pellet and gently resuspend the cells by pipetting up-and-down with a 1 mL micropipette.

Count the cells including a cell viability marker. The typical recovery from one cryovial is $\geq 2 \times 10^6$ viable cells.

7.1.3 Cell Seeding (Day 0):

- 7.1.3.1 Dilute the cell suspension to the required cell concentration using comp:SN+D medium to achieve the required seeding density for your desired experimental conditions. A seeding density of 60,000 cells/cm² is routinely used.

Plate format	Surface (cm ²)	mL/well	Cells/well
6 well	9.5	2.0	570,000
12 well	3.8	1	228,000
24 well	1.9	0.5	114,000
48 well	0.95	0.25	57,000
96 well	0.32	0.1	19,200

- 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% CO₂.
- 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 1 to 7) and Maintenance (Day 7 onwards):

- 7.1.4.1 Day 1 to day 7: Starting from 24h post-thaw, gently perform an 80% medium change daily by gently aspirating 80% of the spent medium and replacing it with fresh pre-warmed comp:SN+D medium (with doxycycline).

Δ Note: Culture of ioSensory 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.

- 7.1.4.2 Day 7: completely replace the culture medium with fresh pre-warmed comp:SN medium (no Doxycycline).
- 7.1.4.3 Day 9: top up the media present in the wells with an additional 100% fresh, pre-warmed comp:SN medium (no doxycycline), for optimal ioSensory neuron maintenance.
- 7.1.4.4 Day 11 onwards: for optimal sensory neuron maintenance, it is recommended a half-medium change regime every 48 hours, i.e., replacing 50% of the medium with fresh comp:SN medium (no Doxycycline).

8. Notes

Technical Support

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