ioGlutamatergic Neurons

Frequently Asked Questions

ioGlutamatergic Neurons
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Frequently Asked Questions
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THE CELL CODING COMPANY

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Shipping, ordering and delivery

1. **What format will the cells be delivered to clients: frozen vials or pre-plated cells?**
   Cells are provided as frozen vials, in either Small (≥0.75.5x10^6 viable cells) or Large (≥1.5x10^6 viable cells) size and shipped in dry ice. They should be stored in liquid nitrogen or ultra-low temperature freezers (-150°C) at recipient's facility immediately until use.

2. **How can I contact you if I have a question?**
   If you have a question regarding bit.bio products or services, you can contact us in the following ways:
   - by website enquiry form: https://bit.bio
   - by email: info@bit.bio / technical@bit.bio
   - by phone: +44 (0) 1223 787 297

Cell revival and experiments

3. **Are ioGlutamatergic Neurons fully differentiated?**
   No, ioGlutamatergic Neurons are not fully differentiated when the end user receives them. ioGlutamatergic Neurons are shipped as 'primed' glutamatergic neurons that have been generated from human pluripotent stem cells at bit.bio using our patented opti-ox cellular reprogramming technology. Cells are delivered in a cryopreserved format and are programmed to rapidly mature upon revival in the recommended medium. The protocol for the generation of these cells is a three-phase process:

   1. Induction (carried out at bit.bio);
   2. Stabilization for 4 days with Doxycycline;
   3. Maintenance during which the glutamatergic neurons mature (Figure 1).

   The cells homogeneously express pan-neuronal markers 2 days post-thawing and require a further 11 days culture to reach functional maturity. Culture protocol conditions are provided in the bit.bio ioGlutamatergic Neurons User Manual.

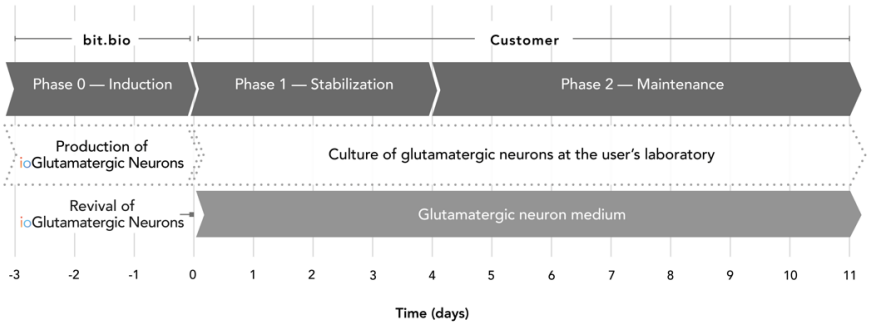

Figure 1
Schematic representation of the three-phase protocol to produce and culture ioGlutamatergic Neurons.

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4. **Can you propagate ioGlutamatergic Neurons once received?**
   ioGlutamatergic Neurons have initiated reprogramming at bit.bio prior to cryopreservation; as such, they cannot be propagated nor passaged further in culture.

5. **What seeding density do you recommend for the ioGlutamatergic Neurons?**
   Neuron cultures are obtained by plating ioGlutamatergic Neurons at a minimum seeding density of 30,000 cells/cm^2. Cells are compatible with plates ranging from 6- to 384-well plate formats. Seeding density may require optimisation depending on the experiment. bit.bio do not advise end users to seed below 30,000 cells/cm^2.

6. **How are cells cultivated?**
   Cells are cultivated in chemically defined culture conditions which are serum free (detailed composition can be found in the User Manual). They are cryopreserved in KnockOut serum replacement (CTS-grade) supplemented by 10% DMSO.

7. **How soon after delivery can ioGlutamatergic Neurons be used for experiments?**
   The table below gives an estimate of the earliest time-points after revival of ioGlutamatergic Neurons at which different assays and experiments have been successfully performed.

Earliest validated time point (days after cell revival)	Validated experiments and assays
Day 1	3D bioprinting
Day 2	scRNA-seq, bulk RNA-seq, ICC, qPCR detecting neuronal markers, compound addition for HCS
Day 3	bDNA detecting neuronal markers
Day 4	qPCR, SCS and RNA-seq detecting glutamatergic and cortical markers
Day 8	Spontaneous neuronal activity detected by MEA
Day 9	HCS assays (HTRF, CTG)
Day 11	ICC detecting glutamatergic markers
Day 13	Synchronised neuronal activity detected by MEA*

* measured in the presence of Astrocytes

bDNA; branch DNA; bulk RNA seq: bulk RNA sequencing; CTG: CellTiter-Glo®; HCS: High Content Screen; HTRF: TR-FRET; ICC: immunocytochemistry; MEA: Multi-Electrode Array; scRNA-seq: single cell RNA sequencing (SCS)

Product information & quality control

8. **Why is opti-ox better than other methods of cellular reprogramming?**
   bit.bio ioGlutamatergic Neurons are derived from human induced pluripotent stem cells (hiPSCs) using proprietary opti-ox technology (as described in Pawlowski et al. 2017), which relies on the precise genetic engineering of hiPSCs with the transcription factor(s) defining a specific cell identity. The opti-ox system enables unprecedented batch-to-batch

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reproducibility, homogeneity of differentiation and scalability compared to classical approaches using lentiviral vectors. ioGlutamatergic Neurons are easy to culture and within days of revival convert into pure, mature and functional glutamatergic neurons. The table below compares bit.bio's opti-ox technology to other competitor products available on the market.

Parameter	ioGlutamatergic Neurons	Competitor glutamatergic neurons
Cell culture phenotype	>80% glutamatergic neurons alongside a minor Cholinergic subpopulation	>60% glutamatergic neurons together with GABAergic and Cholinergic subpopulations
Ease of use	• 1-step coating of vessels with GelTrex • Open source: defined medium supplements	• 3-step coating: 1. Poly-L-Ornithine; 2. 3x PBS wash; 3. Matrigel coating • Proprietary: undisclosed media supplements provided by the manufacturer
Recommended seeding density	30,000 cells/cm^2	250,000 cells/cm^2
Number of cells required to plate a 96-well plate culture	922 x10^3 cells	7680 x10^3 cells
Number of cells required to plate a 384-well plate culture	645 x10^3 cells	5376 x10^3 cells

9. **What were the cells of origin for ioGlutamatergic Neurons?**
   ioGlutamatergic Neurons are generated from hiPSCs. The parental iPSC line has been derived from Caucasian white male dermal fibroblasts using the four retrovirally transduced Yamanaka factors (OCT4, SOX2, KLF4, MYC).

10. **Do you have donor consent for the parental hiPSCs?**
    All of the cells used by bit.bio have been derived under approved ethical agreement from voluntary donors who have signed an informed consent which outlines the purpose of the donation. If you require more information, please contact info@bit.bio. The Statement of Use can be accessed here: https://bit.bio/statement-of-use.pdf

11. **Do you use viral vectors to manufacture ioGlutamatergic Neurons?**
    No, only recombinant DNA vectors are used to generate ioGlutamatergic Neurons from the parental hiPSC line*.

    * However, replication deficient retroviral vectors (non-infectious) have been used for the reprogramming of the parental hiPSC line (characterised master line from dermal fibroblasts).

12. **What is the host and transgene used to generate cells?**
    The host is human and the transgene used to differentiate the hiPSCs towards ioGlutamatergic Neurons is human NEUROG2 coding sequence. Note that the cells also express additional transgenes that are an integral part of the opti-ox system: PAC (puromycin resistance; prokaryote), NEO (neomycin resistance; prokaryote) and rtTA (TetON system; prokaryote).

13. **What substances other than KnockOut serum may be present in the freezing medium?**
    Cells are cryopreserved in KnockOut serum replacement (CTS-grade) supplemented with 10% DMSO. Please refer to the table below for all

compounds used for the manufacture of ioGlutamatergic Neurons that may be carried over in the freezing medium.

Reagent	Supplier	Cat. Number	Storage
ROCKi (Y-27632)	Stratech Scientific	S1049-SEL	-20°C to -80°C
Geltrex (Reduced GF)	ThermoFisher	A1413202	-20°C to -80°C
DMEM/F-12	ThermoFisher	11330032	2°C to 8°C
Neurobasal	ThermoFisher	21103049	2°C to 8°C
B27	ThermoFisher	17504044	-20°C to -80°C
Glutamax	ThermoFisher	35050061	2°C to 8°C
2-Mercaptoethanol	ThermoFisher	31350010	2°C to 8°C
NT3	R&D	267-N3-025	-20°C to -80°C
BDNF	R&D	248-BDB-005	-20°C to -80°C
Doxycycline	Sigma	D9891	2°C to 8°C
Bovine Serum Albumin	Sigma	A7906	-20°C to -80°C

14. **What quality control is performed on the ioGlutamatergic Neurons?**
ioGlutamatergic Neurons production batches are tested for sterility, viability and maturity acquisition over time by monitoring the expression of key genes by RT-qPCR: pan-neuronal (SYP, TUBB3), glutamatergic neuron-specific (VGLUT2), glutamatergic receptor (GRIA4) as well as loss of pluripotency genes (OCT4, NANOG). ioGlutamatergic Neurons culture purity is further checked by immunofluorescent staining for pan-neuronal proteins (TUBB3, MAP2) and glutamatergic neuron-specific transporters (VGLUT1, VGLUT2).

15. **How does bit.bio confirm its cell lines are free from contamination?**
We follow strict aseptic bio-banking procedures and each manufactured cell lot is tested for sterility (microbial and fungal) and absence of mycoplasma infection (pan species) by industry standard validated means, post-thawing.

16. **Can you please provide some references for the ioGlutamatergic Neurons?**
Selected publications that describe ioGlutamatergic Neurons cells include:
    - Pawlowski, M. et al. (2017). Inducible and Deterministic Forward Programming of Human Pluripotent Stem Cells into Neurons, Skeletal Myocytes, and Oligodendrocytes. Stem Cell Report, 8, 803-812.
    - Tourigny, D. S. et al. (2019). Energetic substrate availability regulates synchronous activity in an excitatory neural network. PLOS ONE, 14(8), e0220937.
    - Zhou, L. et al. (2020) Lipid-Bilayer-Supported 3D Printing of Human Cerebral Cortex Cells Reveals Developmental Interactions. Advanced Materials, 32:200218.

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