ioGlutamatergic Neurons

HTT·50CAG/WT

Frequently Asked Questions

ioGlutamatergic Neurons
HTT $^{50CAG/WT}$
Catalogue No: ioEA1004

Frequently Asked Questions
Document NPI-0033 FAQ V-01

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Shipping, ordering and delivery

1. **In what format will the cells be delivered: frozen vials or pre-plated cells?**
   Cells are provided as frozen vials, in either Small (≥1x10^6 viable cells) or Large (≥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 the 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

1. **Are ioGlutamatergic Neurons HTT$^{50CAG/WT}$ fully differentiated?**
   No, ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are not fully differentiated when received by the end user.

   ioGlutamatergic Neurons HTT$^{50CAG/WT}$ 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. Stabilisation 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 HTT$^{50CAG/WT}$ User Manual.

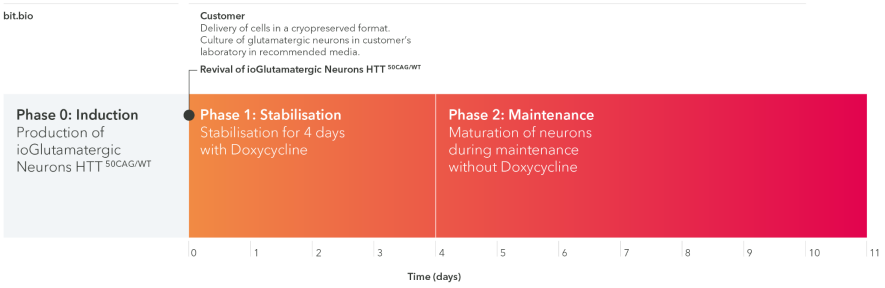

Figure 1
Schematic representation of the three-phase protocol to culture ioGlutamatergic Neurons HTT$^{50CAG/WT}$.

2. **Can you propagate ioGlutamatergic Neurons HTT$^{50CAG/WT}$ once received?**
   ioGlutamatergic Neurons HTT$^{50CAG/WT}$ already began the process of maturing into neurons at bit.bio prior to cryopreservation. They are post-mitotic when you receive them, and as such, they cannot be propagated nor passaged further in culture.

3. **What ioGlutamatergic Neurons HTT$^{50CAG/WT}$ seeding density do you recommend for plating?**
   Neuron cultures are obtained by plating ioGlutamatergic Neurons HTT$^{50CAG/WT}$ 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 will require optimisation depending on the experiment, different assays may require higher seeding densities. We do not advise end users to seed below 30,000 cells/cm^2.

4. **How are the ioCells cultivated?**
   ioCells 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.

5. **How soon after delivery can ioGlutamatergic Neurons HTT$^{50CAG/WT}$ be used for experiments?**
   The table below gives the earliest time-points after revival of wild type ioGlutamatergic Neurons (io1001) at which different assays and experiments have been successfully performed.

   Note: This is the parent line of ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the timing is likely to be closely aligned.

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)

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Product information & quality control

1. **Why is opti-ox™ better than other methods of cellular reprogramming?**
   ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are derived from human induced pluripotent stem cells (hiPSCs) using proprietary opti-ox^{TM} 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 reproducibility, homogeneity of maturation, and scalability compared to classical approaches using lentiviral vectors. ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are easy to culture and within days of revival convert into pure, mature and functional Huntington's disease cell models. The table below compares bit.bio's opti-ox technology in the wild type ioGlutamatergic Neurons (io1001) 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	2-step coating of vessels with PDL and 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	ranging between 100,000 - 250,000 cells/cm^2
Number of cells required to plate a 96-well plate culture	922 x10^3 cells	4608 x10^3 cells
Number of cells required to plate a 384-well plate culture	645 x10^3 cells	3226 x10^3 cells

2. **What were the cells of origin for ioGlutamatergic Neurons HTT$^{50CAG/WT}$?**
   ioGlutamatergic Neurons HTT$^{50CAG/WT}$ 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).

3. **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

4. **Do you use viral vectors to manufacture ioGlutamatergic Neurons HTT$^{50CAG/WT}$?**
   No, only recombinant DNA vectors are used to generate ioGlutamatergic Neurons HTT$^{50CAG/WT}$ 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).

5. **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 HTT$^{50CAG/WT}$ is a human 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).

6. **How was the 50 CAG repeat expansion introduced to the cells and how was the mutation verified?**
   A plasmid donor containing left and right arms of homology, and the 50 CAG repeat template was targeted to exon 1 of the *HTT* gene using CRISPR/Cas9 delivered by RNP. PCR and gel electrophoresis were used to confirm the integration of the 50 CAG expansion in one *HTT* allele, and no random integration of the plasmid in the genome. NGS-amplicon sequencing confirms the presence of both the wild type and 50 CAG alleles. The number of CAG repeat reads peak at 24 in the wild type allele and 50 in the mutant allele.

7. **Have you assessed the karyotype of these cells?**
   Yes, the ioGlutamatergic Neurons HTT$^{50CAG/WT}$ cells showed no chromosomal abnormalities when analysed by KaryoStat^{TM} assay and G-banding.

8. **What quality control is performed on the ioGlutamatergic Neurons HTT$^{50CAG/WT}$?**
   ioGlutamatergic Neurons HTT$^{50CAG/WT}$ production batches are tested for sterility, viability, morphology, and maturity acquisition over time by monitoring the expression of key genes by RT-qPCR: pan-neuronal (SYP, TUBB3), glutamatergic neuron-specific (VGLUT1 and VGLUT2), glutamatergic receptor (GRIA4) as well as loss of pluripotency genes (OCT4, NANOG). ioGlutamatergic Neurons HTT$^{50CAG/WT}$ culture purity is further checked by immunofluorescent staining for pan-neuronal proteins (TUBB3, MAP2) and glutamatergic neuron-specific transporters (VGLUT2).

9. **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.

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10. **Can you please provide some references for the ioGlutamatergic Neurons HTT$^{50CAG/WT}$?**

    Currently, no published research exists that includes data from ioGlutamatergic Neurons HTT$^{50CAG/WT}$. However, selected publications that describe the wild type parent line of ioGlutamatergic Neurons HTT$^{50CAG/WT}$ (io1001) 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.st
    - Pavelinek, A. et al. (2021). Interferon-ɣ Exposure of Human iPSC-derived Neurons Alters Major Histocompatibility Complex I and Synapsin I Protein Expression. bioRxiv.*

    * The research in this paper was conducted with the early development cell line, not the final bit.bio product.

11. **Can the wild type ioGlutamatergic Neurons be used as a control for ioGlutamatergic Neurons HTT$^{50CAG/WT}$?**

    Yes, the ioGlutamatergic Neuron HTT$^{50CAG/WT}$ Disease Model and the wild type ioGlutamatergic Neurons (io1001) were derived from the same parental iPSC cell line. A plasmid donor with a 50 CAG repeat expansion was introduced into one allele of the *HTT* gene in the wild type ioGlutamatergic Neuron cell line, using CRISPR/Cas9 gene editing. We recommend using the wild type ioGlutamatergic Neurons in your experiment as a genetically matched control.

12. **How have you ensured that there are no off-target effects from introducing the disease mutation into the ioGlutamatergic Neurons HTT$^{50CAG/WT}$?**

    The integration of the *HTT* 50 CAG mutation has been verified to be on-target and in one allele by PCR and gel electrophoresis. The image below shows the on-target integration of 50 CAG repeat expansion into one *HTT* allele (left panel). Amplicon PCR of the plasmid donor reveals no random integration in genomic DNA from targeted colonies (right panel) via gel electrophoresis.

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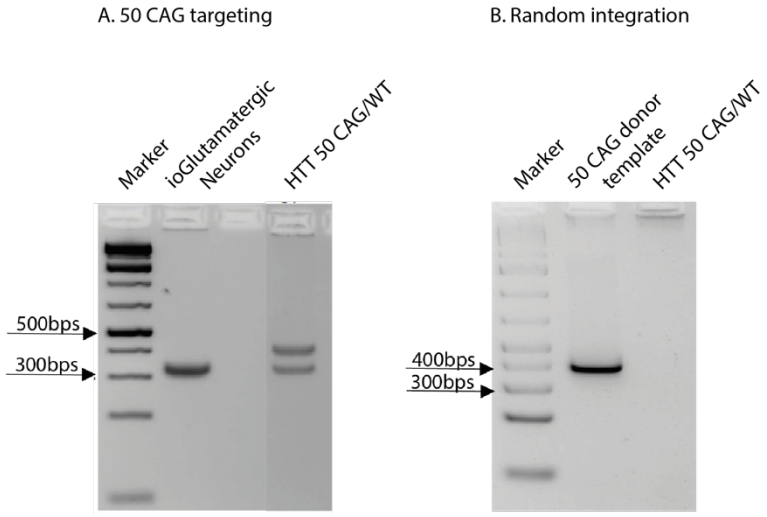

Figure 1

The CAG repeat expansion was successfully integrated into one *HTT* allele, as confirmed by Gel Electrophoresis.

A) Confirmation of the on-target integration of a 50 CAG repeat expansion into one *HTT* allele. Genotyping primers flanking the endogenous *HTT* CAG repeat region produce a band at approximately 320 base pairs by PCR. Bands at 395 base pairs detect on-target gene editing and the introduction of a 50 CAG repeat. **B**) Amplicon PCR of the donor plasmid backbone reveals no random integration of the 50 CAG repeat expansion into the genome of targeted colonies via gel electrophoresis.

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