ioGlutamatergic Neurons

Digital flyer
Cat. No io1001
(formerly e001)

bit.bio
THE CELL CODING COMPANY

Discovery accelerated with reliable, scalable and functional human excitatory neurons

ioGlutamatergic Neurons have been reprogrammed from human induced pluripotent stem cells (iPSCs) using our precise reprogramming technology: opti-ox™ [1] (optimized inducible overexpression). Human stem cells, within days, convert into consistent, mature, functional glutamatergic neurons providing a high quality human model for the study of neurological activity and disease.

1. Pawlowski et al. Stem Cell Reports 2017
www.ncbi.nlm.nih.gov/pmc/articles/PMC5390118

Characteristics that inspire confidence

Defined
Characterised by ICC and gene expression.

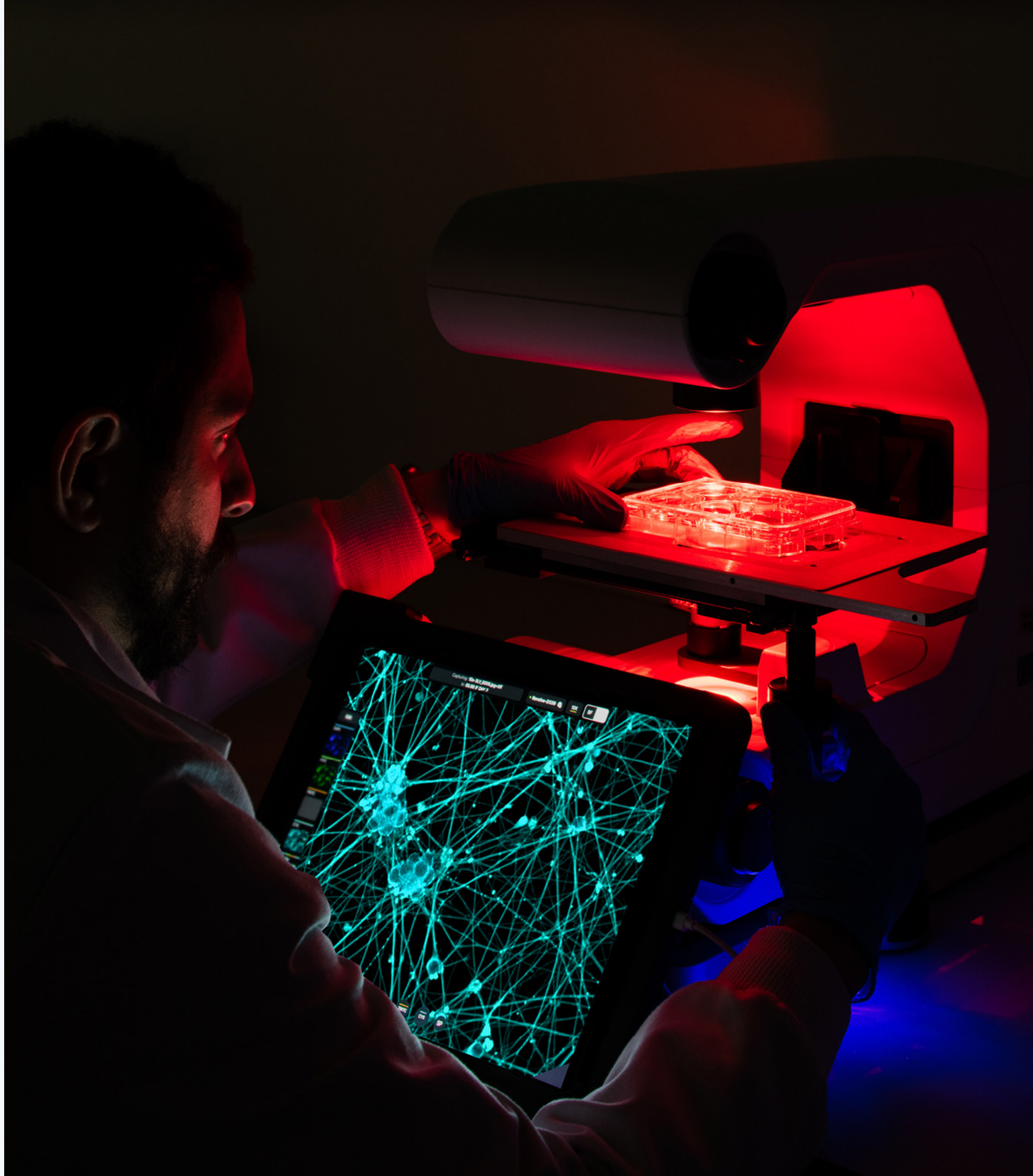
Scalable
Industrial scale quantities at a price point that allows the cells to be used from research to screening scale.

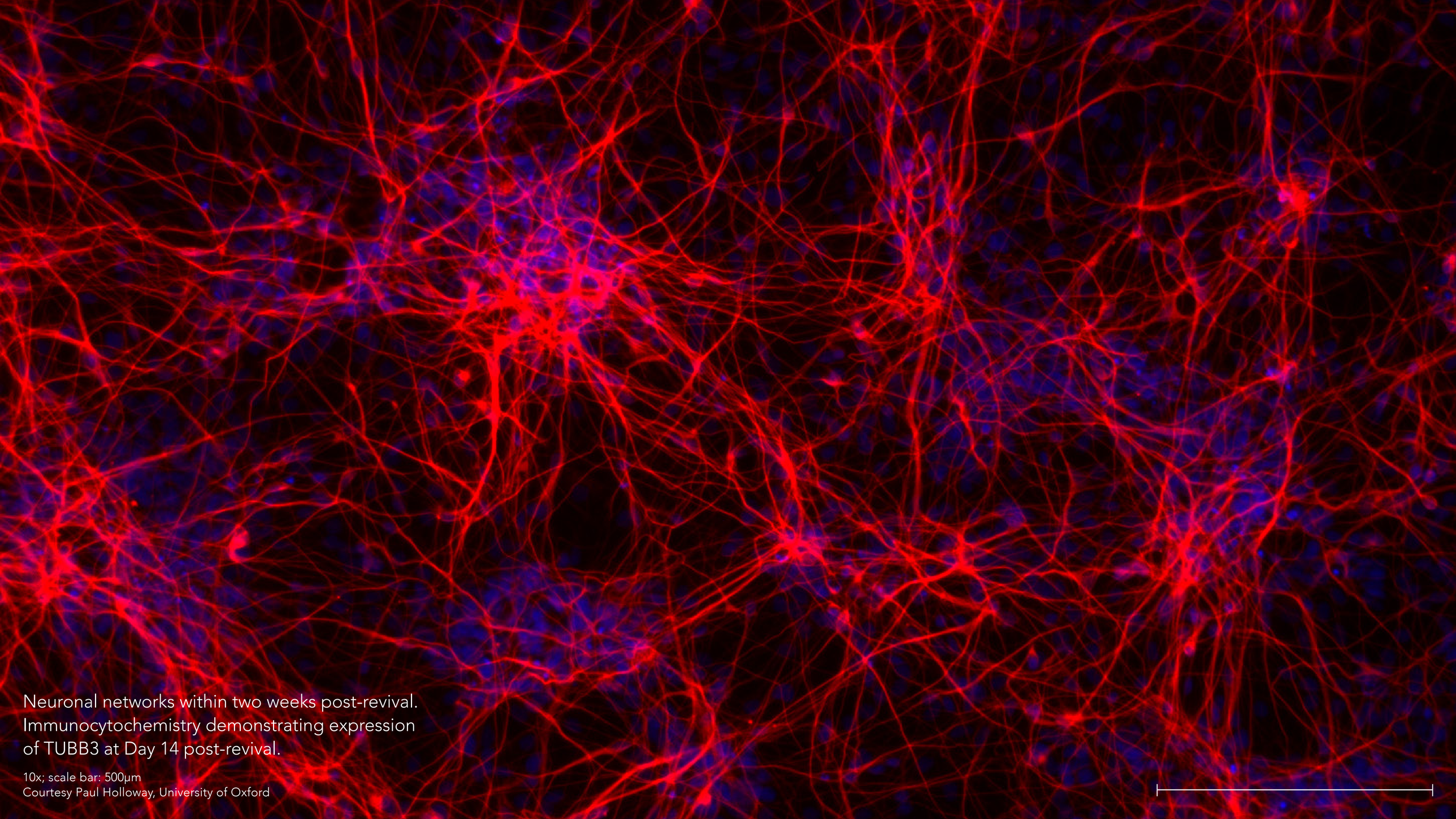
Consistent
Batch to batch reproducibility and homogeneity create a stable human model for excitatory neuronal activity and disease.

Quick
Spontaneous electrical activity observed within 8 days post revival, and functional neuronal networks observed within 13 days.

Easy-to-use
Cells arrive programmed to rapidly mature upon revival. One medium required in a two-step protocol.

Cost-effective
Available in two vial sizes, tailored to suit your experimental needs with minimal waste.





Neuronal networks within two weeks post-revival. Immunocytochemistry demonstrating expression of TUBB3 at Day 14 post-revival.

10x; scale bar: 500µm
Courtesy Paul Holloway, University of Oxford

Mature neuronal properties within two weeks of induction

Express glutamatergic neuron-specific markers & exhibit neurite outgrowth

ioGlutamatergic Neurons are derived from human iPSCs by Neurogenin-2 (NEUROG2) driven opti-ox™ reprogramming, resulting in the rapid differentiation of stem cells into mature and functional neurons.

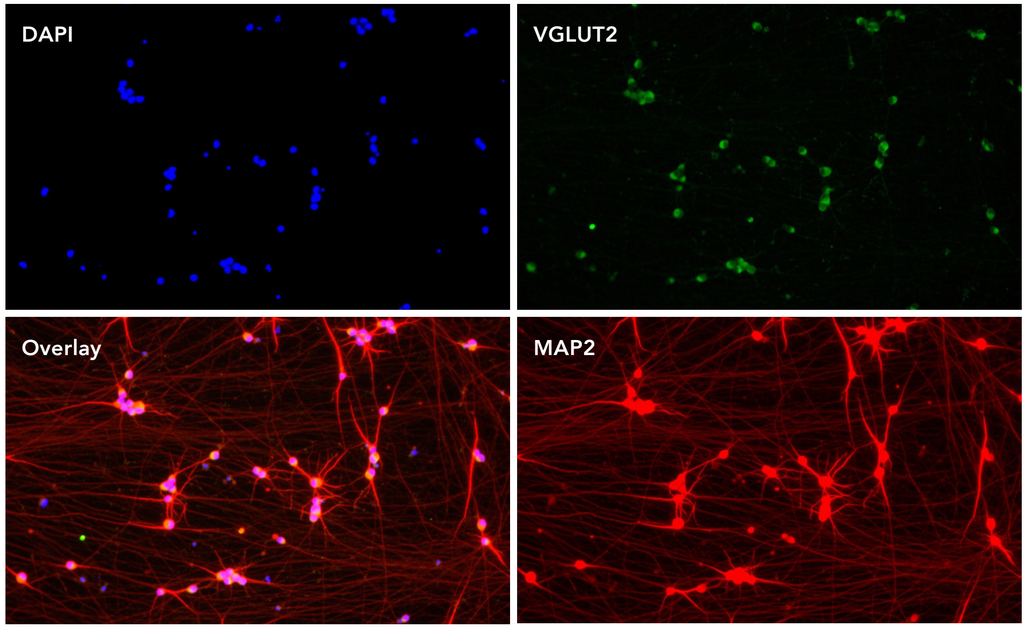
Cultures consist mainly of glutamatergic neurons (>80%) characterised by homogenous expression of pan-neuronal proteins MAP2 and TUBB3, and glutamatergic-specific transporter VGLUT2. The minor remaining fraction of the neuronal population express marker genes of cholinergic neurons.

A bulk RNA-seq analysis shows that ioGlutamatergic Neurons have a rostral CNS identity and express the classical cortical marker genes FOXG1 and TBR1 (data not shown).

“ These cells provide a reliable and pure source of glutamatergic neurons, resembling primary human ones. They are ready-to-use which makes it much more easy for tissue culture work and for reproducible results.”

Dr Koby Baranes, University of Cambridge

Immunocytochemistry at Day 11 post-revival



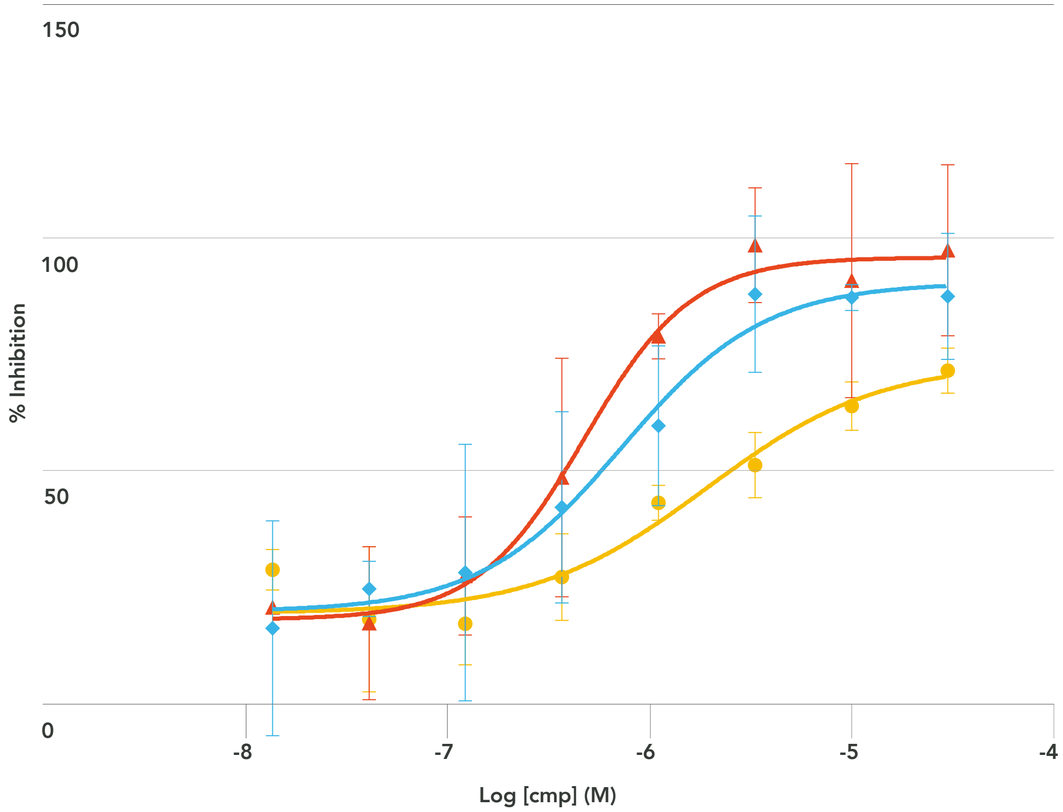
Robust and scalable cells for high-throughput applications

Validated for CellTiter-Glo® and HTRF® assays

Cytotoxicity CellTiter-Glo® (CTG) and TR-FRET (HTRF®) assays for AKT serine/threonine kinase 1 (AKT) and Huntingtin (HTT) proteins were performed on ioGlutamatergic Neurons in 384-well plates treated with tool compound (cmp) at day 9 post-revival. Compound titration results in a concentration response curve for all three assays (mean±sd of 2 replicates).

CTG assay on ioGlutamatergic Neurons shows an excellent average signal/background ratio and high suitability for HTS. HTRF® assays on ioGlutamatergic Neurons show lower signals but with low variability, and could therefore also provide a suitable platform for HTS.

Iovino M., et al., 2019, Charles River Laboratories



CTG
HTT
AKT

Signal/Background	
CellTiter-Glo®	327
HTT HTRF®	1.65
AKT HTRF®	1.95

Neuronal activity that matures over-time

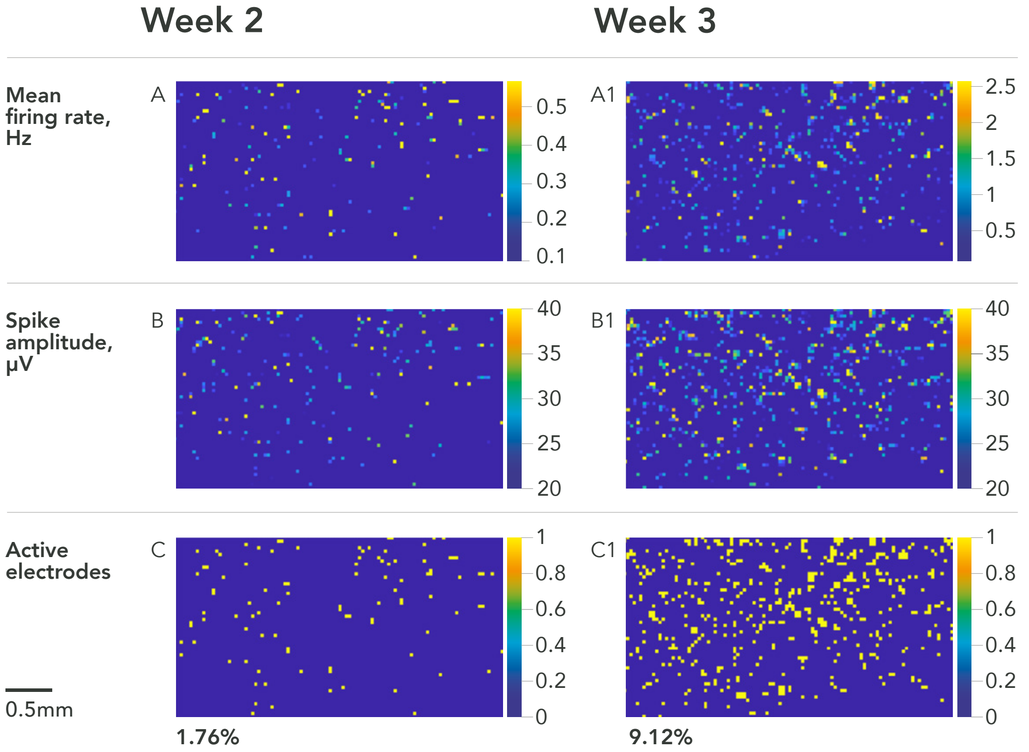
Examples of MaxOne high-resolution multi-electrode array (MEA) recordings of ioGlutamatergic Neurons in BrainPhys™ media

The activity maps show firing rate (A), spike amplitude (B) and % of active electrodes (C). Results demonstrate a time-dependent increase of spontaneous activity during neuronal maturation from 2 to 3 weeks post-revival.

Iovino M., et al., 2019, Charles River Laboratories

"These cells enable us to move rapidly as from the moment of plating within 4–7 days we have mature and functional neurons."

Dr Shushant Jain, Charles River



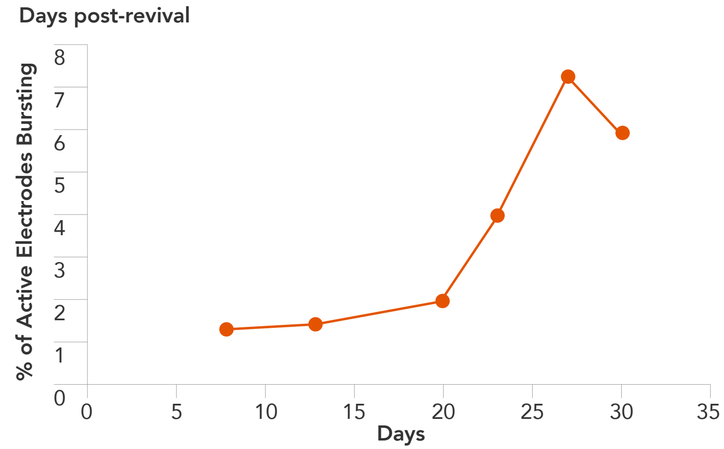
Rapid gain of functional activity

Time-dependent increase of spontaneous bursting activity over a three week period

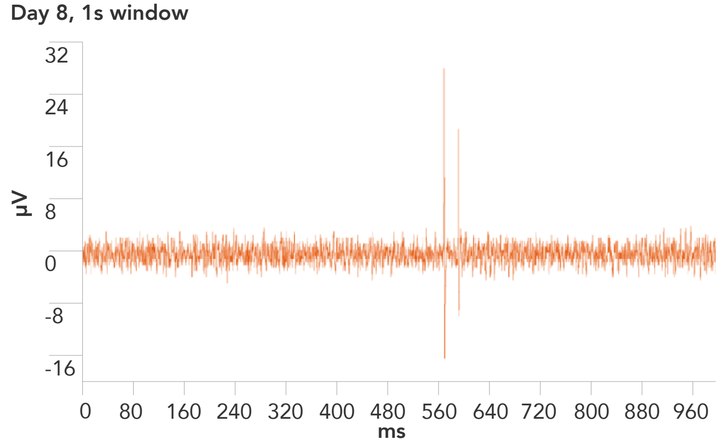
A. The graph shows the % of active bursting electrodes for each time point.
B. An example of a spontaneous spike, taken at Day 8 post-revival (1 second sweep, 32 µV/-18 µV).
C. An example of a bursting phenotype, taken at Day 20 post-revival (1 second sweep, 16 µV/-16 µV). Cells were cultured in the standard user manual medium (Complete Glutamatergic Neuron Medium) and recorded on 64-electrode MEA (Multi Channel Systems).

NDimension (Science and Engineering) Ltd

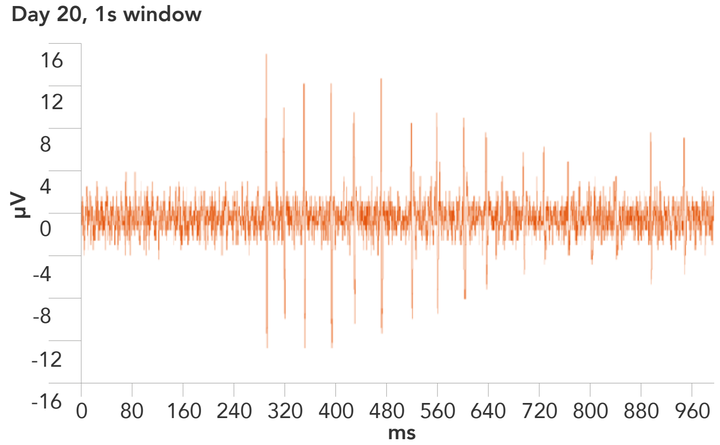
A
Percentage of active electrodes bursting



B
Example of a spontaneous spike



C
Example of a bursting phenotype

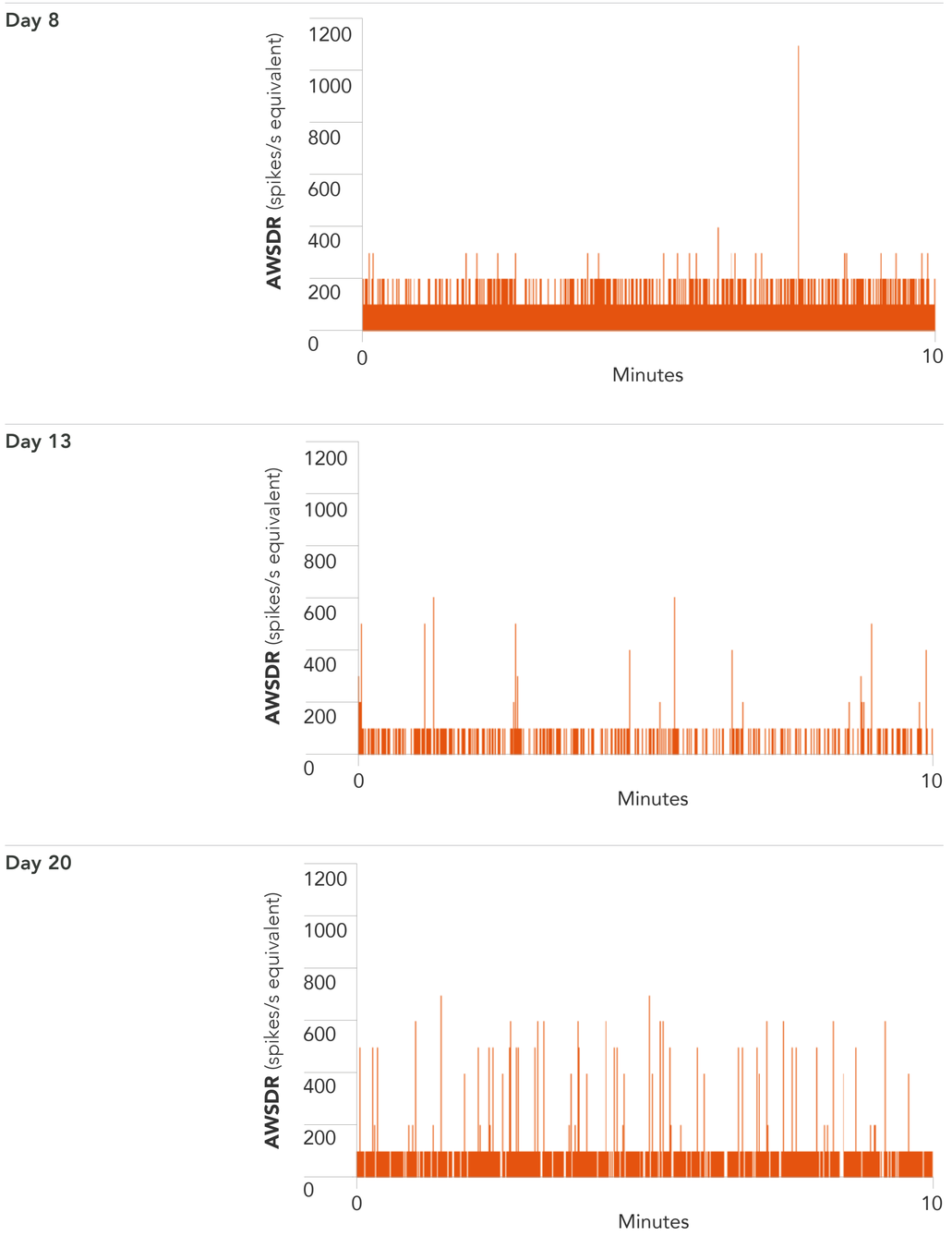


ioGlutamatergic Neurons co-cultured with rat-derived astrocytes demonstrate a time-dependent increase in synchronous activity

Array Wide Spike Detection Rate histograms (AWSDR – a graphical measure of synchrony) for 10-minute recordings on Day 8, 13 and 20 post-revival, in co-culture with primary rat-derived astrocytes

Results show prominent synchronicity on Day 13, exemplified by the 'spikier' nature of the associated AWSDR, which increases at Day 20. Cells were cultured in the standard user manual medium (Complete Glutamatergic Neuron Medium) and recorded on 64-electrode MEA (Multi Channel Systems).

NDimension (Science and Engineering) Ltd



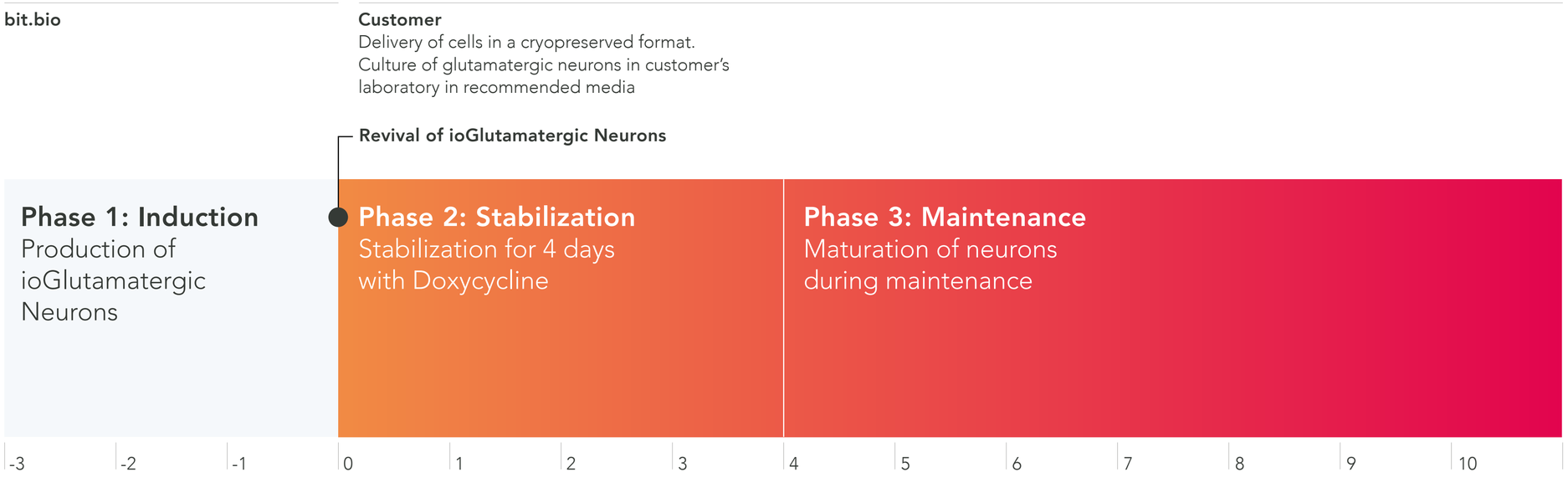
Ready-to-culture and easy-to-use

ioGlutamatergic Neurons are delivered in a cryopreserved format and are programmed to rapidly mature upon revival

Cells are kept in a single culture medium formulation from revival with fully disclosed composition allowing modifications to fit customer's bespoke experiments. 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 neurons mature

> **"These cells were really easy-to-use and ready to be plated in the assay format."**
>
> Dr Mariangela Iovino, Charles River



Cost effective and flexible

ioGlutamatergic Neurons are compatible with plates ranging from 6 to 384 wells and are available in two vial sizes, tailored to suit your experimental needs with minimal waste

Recommended seeding density is 30,000 cells/cm^2.

One Small vial can plate a minimum of 0.5 x 24-well plate, 0.75 × 96-well plate, or 1 x 384-well plate.

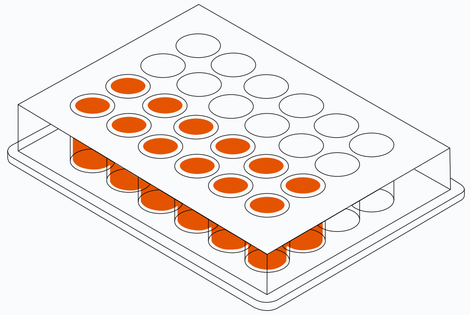
One Large vial can plate a minimum of 1 x 24-well plate, 1.5 × 96-well plate, or 2 x 384-well plates.

Recommended seeding density

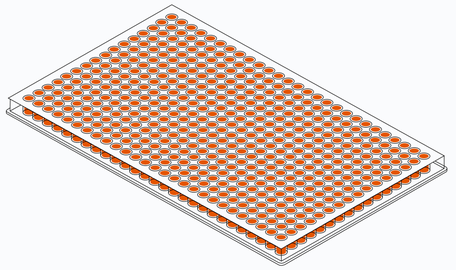
30,000 cells/cm^2

Small vial size

0.75 × 10^6 cells



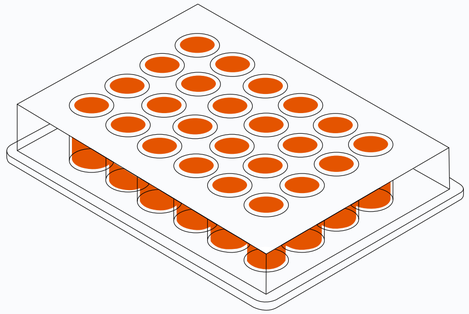
24-well plate



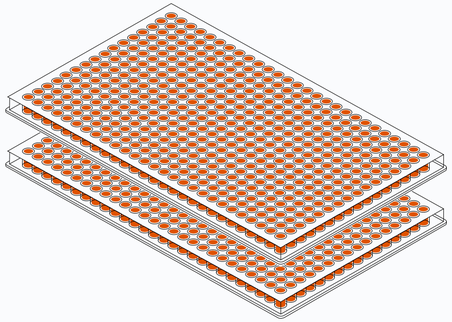
384-well plate

Large vial size

1.5 × 10^6 cells



24-well plate



384-well plate

Upcoming products

Our range is constantly expanding. Coming soon are iPSC-derived inhibitory ioGABAergic Neurons and neuronal cells with disease-relevant mutations for modelling Huntington's, Parkinson's, Frontotemporal Dementia and ALS.

Request an invite to be the first to know about our latest products by emailing info@bit.bio.

Contact us

To order or speak with a member of our team, email orders@bit.bio.

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Product specificiations

Starting material
Human iPSC line

Donor
Caucasian adult male (skin fibroblast)

Differentiation method
opti-ox™ cellular reprogramming

Karyotype
Normal (46, XY)

Vial size
Small: >0.75 × 10^6 viable cells
Large: >1.5 × 10^6 viable cells

Recommended seeding density
30,000 cells/cm^2

Seeding compatibility
6 to 384 well plates

Quality control
Sterility, protein expression (IF) and gene expression (RT-qPCR)

User storage
LN2 or -150°C

Shipping info
Dry ice

Product use
These cells are for research use only

Product applications

- **Academic research**
- **Drug development**
- **Neurotoxicology**
- **High-throughput screening**
- **Genetic screening (e.g. CRISPR screening)**

Validated techniques include
- HTRF® and CellTiter-Glo® assays
- RT-qPCR and branch DNA
- Immunocytochemistry assays (ICC)
- Patch clamp and multi-electrode array (MEA)
- Bulk & single cell RNA seq
- Co-culture
- 3D bioprinting

HTRF® is the registered trademark of Cisbio. CellTiter-Glo® is the registered trademark of Promega.

Pricing

Size	Price
Small	£1,199.00
Large	£1,799.00
Multipack (4 Large)	£5,600.00

Please contact us for academic and non-profit discount