CRISPR-Ready ioGlutamatergic Neurons™

Human iPSC-derived glutamatergic neurons expressing Cas9 for rapid gene knockout generation

Learn more about CRISPR-Ready ioGlutamatergic Neurons

ioCells™

About the cells

CRISPR-Ready ioGlutamatergic Neurons are precision reprogrammed iPSC-derived neurons containing a constitutively expressed Cas9 nuclease. This product has been designed for scientists looking to generate gene knockouts in a physiologically relevant human cell. These cells arrive ready for guide RNA (gRNA) delivery by day 1 post-thaw. Using our optimised lentivirus or lipid-based gRNA delivery protocol, users can maximise their knockout efficiency and start measuring readouts from gene knockouts and CRISPR screens within days.

Benchtop benefits

READY-TO-USE

Defined and characterised human neurons constitutively expressing Cas9, ready for knockout experiments from day 1.

QUICK AND EASY

Generate readouts within days using a simple protocol for cell maturation and gRNA delivery.

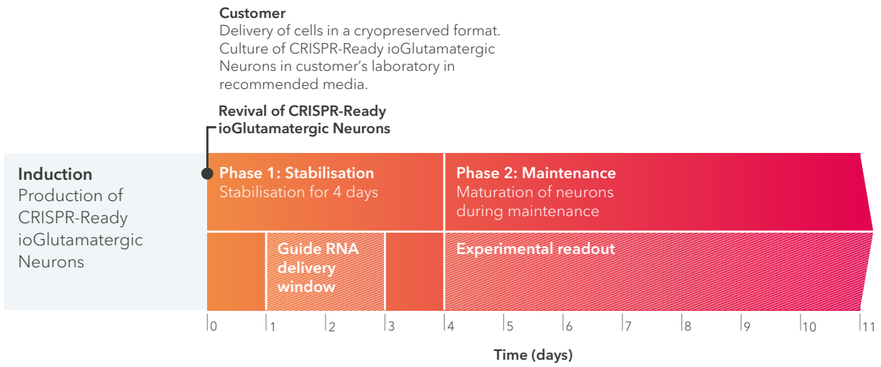
HIGH KNOCKOUT EFFICIENCY

Optimised protocols for lipid or lentivirus based gRNA delivery ensure maximal knockout efficiency.

Go from seeding to knockout to readout in days

CRISPR-Ready ioGlutamatergic Neurons arrive ready to use. With our optimised lentivirus or lipid-based guide delivery protocols, users can cut experimental timelines to a matter of days by no longer needing to invest time engineering and characterising their own Cas9 stable iPSC lines or optimising iPSC differentiation protocols.

With these cells, robust experimental readouts can be achieved by simply delivering gRNA against your target gene. Users do not require prior expertise in iPSC differentiation or gRNA delivery optimisation.

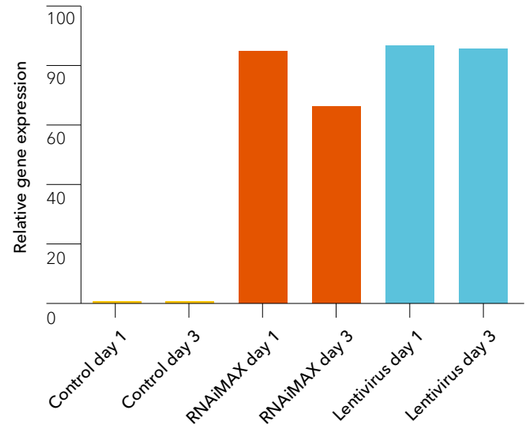


High-efficiency single-gene knockouts can be achieved with both lentiviral and lipid-based gRNA delivery using CRISPR-ready ioGlutamatergic Neurons, observed by amplicon sequencing

Neurons at scale for CRISPR screens

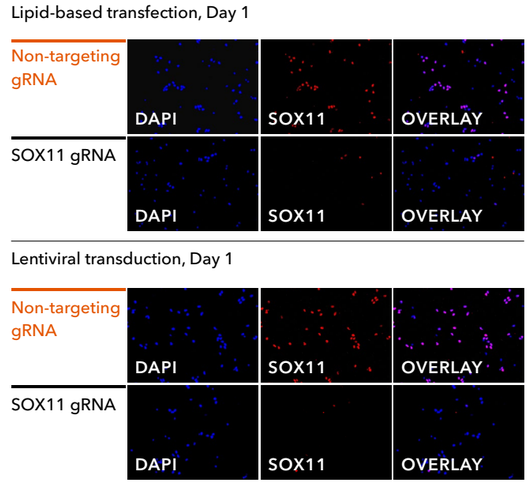
High-efficiency single-gene knockouts can be achieved with both lentiviral and lipid-based gRNA delivery using CRISPR-ready ioGlutamatergic Neurons, observed by amplicon sequencing

SOX11 indel formation was measured by amplicon sequencing in CRISPR-Ready ioGlutamatergic Neurons transfected or transduced with a gRNA targeting SOX11 delivered at either day 1 or day 3 after thawing. gRNAs targeting SOX11 were delivered by either lentiviral transduction or as a synthetic gRNA (10 µM) using Invitrogen™ Lipofectamine™ RNAiMAX transfection reagent. DNA was harvested from the cells after 3 days of culture post transduction or transfection for amplicon sequencing of SOX11. Comparable knockout efficiencies were achieved for both lentiviral transduction and lipid-based transfection.



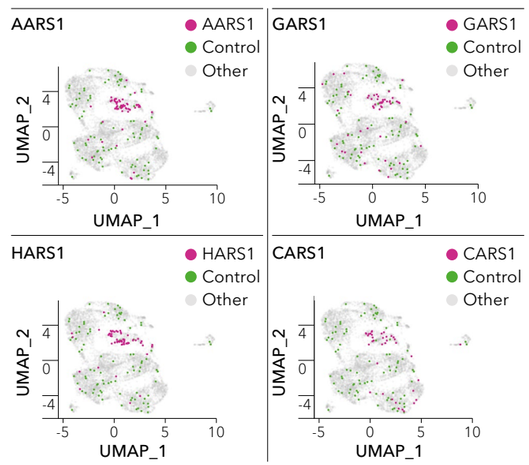
High knockout efficiencies have been validated at the protein level by immunofluorescent staining

Immunofluorescent staining on CRISPR-Ready ioGlutamatergic Neurons transfected or transduced with a gRNA targeting SOX11 shows high percentage of knockout cells. gRNAs targeting SOX11 were delivered by either lentiviral transduction or as a synthetic gRNA (10 µM) using Invitrogen Lipofectamine RNAiMAX transfection reagent at day 1 or day 3 (data not shown) after thawing. Cells were fixed for SOX11 immunofluorescence staining after three days of culture post-transduction or transfection. Comparable knockout efficiencies were achieved for both lentiviral transduction and lipid-based transfection.100X magnification.



CRISPR-ready ioGlutamatergic neurons can be scaled to CRISPR screening workflows, and used to identify disease-relevant targets

A pooled single-cell CRISPR screen was designed to study the transcriptomic effects of knocking out 100 disease-relevant genes in CRISPR-ready ioGlutamatergic Neurons. Lentiviral gRNA transduction was carried out at day 3, and single cell gene expression analysis was performed at day 12. Cells that received non-targeting control gRNA (green dots) are evenly distributed across the UMAP, meaning no significant transcriptomic effects were observed. Cells that received gRNA targeting aminoacyl-tRNA synthetase (aaRSs) genes (purple dots) clustered together, indicating similar transcriptomic profiles. Pathway analysis of this cluster showed activation of the unfolded protein response, a pathway of therapeutic significance in Charcot–Marie–Tooth disease



Product information

Cat code
ioEA1090

Starting material
Human iPSC line

Karyotype
Normal (46, XY)

Seeding compatibility
6, 12, 24, 48, 96 & 384 well plates

Shipping info
Dry ice

Donor
Caucasian adult male
(skin fibroblast)

Vial size
Small: >1 x 10^6 viable cells

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

Differentiation method
opti-ox™ cell reprogramming

Recommended seeding density
30,000 cells/cm^2

User storage
LN2 or -150°C

Format
Cryopreserved cells

Product use
ioCells™ are for
research use only

Applications
Single gene knockouts, Combinatorial gene knockouts, Pooled CRISPR screens, Arrayed CRISPR screens, Drug discovery, High throughput screening

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THE CELL CODING COMPANY

Who we are

bit.bio combines the concepts of cell programming and biology to provide human cells for research, drug discovery and cell therapy, enabling a new generation of medicines.

This is possible with our precision human cellular reprogramming technology opti-ox™ – a gene engineering approach that enables unlimited batches of any human cell to be manufactured consistently at scale

For general information,
email info@bit.bio

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