ioSkeletal Myocytes

Digital flyer
Cat. No io1002
(formerly EA1200)

bit.bio
THE CELL CODING COMPANY

Accelerate your research with rapidly maturing and contractile human muscle cells that are ready for experimentation in days

Introducing highly-defined, consistent and reliable human muscle cells for research, disease modelling and high throughput screening focused on muscle, neuromuscular, and associated metabolic disorders.

ioSkeletal Myocytes have been reprogrammed from human iPSCs by MYOD1-driven opti-ox™ [1] reprogramming. The technology allows for human pluripotent stem cells, within days, to convert into striated, multinucleated myocytes that contract in response to acetylcholine by Day 10 post-revival.

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.

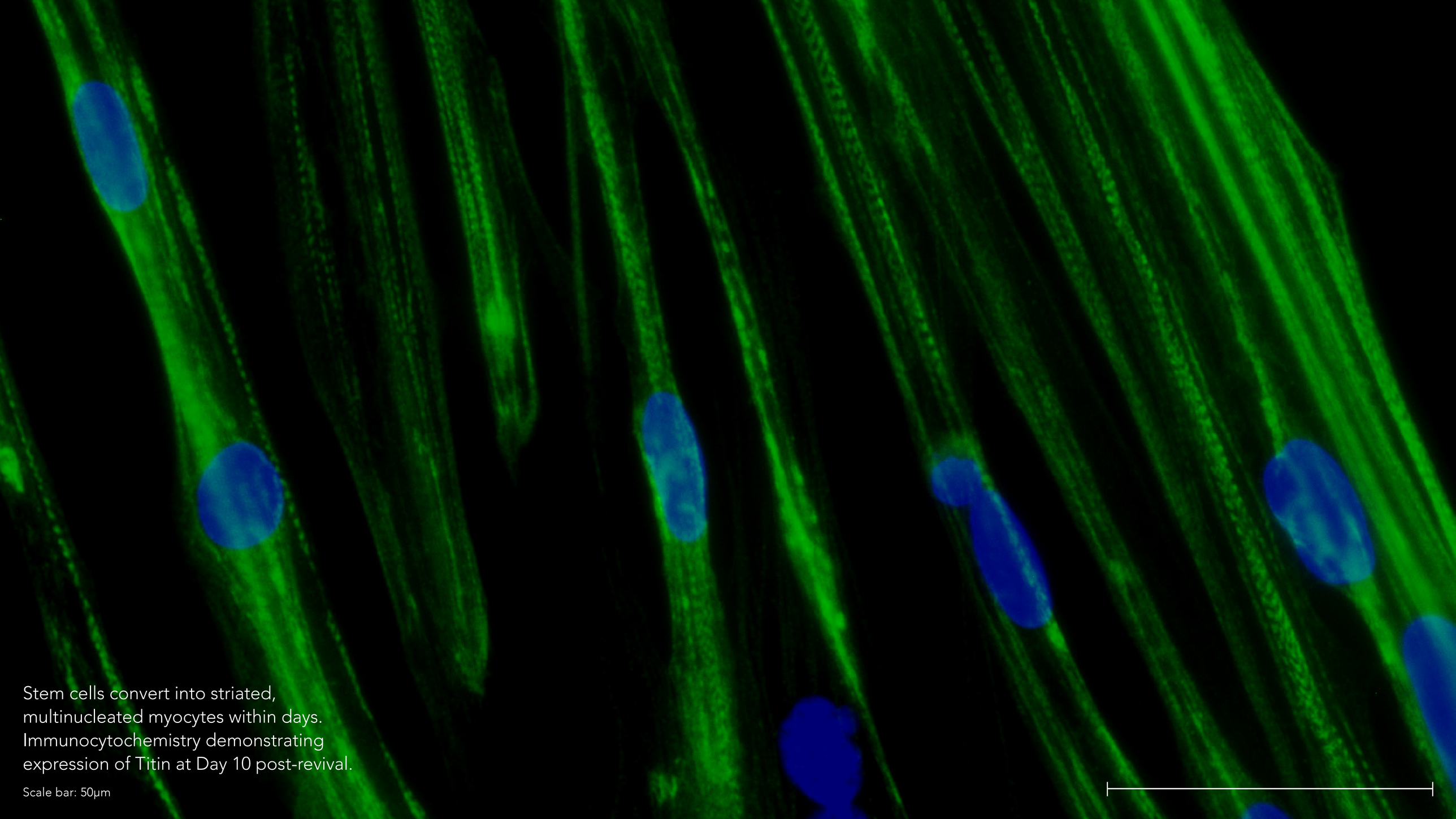
Scalable
Industrial scale quantities at a price point that allows the cells to be used from research to screening scale.

Consistent
Batch to batch reproducibility and homogeneity create a stable human model for the study of muscle, neuromuscular, and associated metabolic disorders.

Quick
Ready for experimentation within days post-revival.

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.



Stem cells convert into striated, multinucleated myocytes within days. Immunocytochemistry demonstrating expression of Titin at Day 10 post-revival.

Scale bar: 50µm

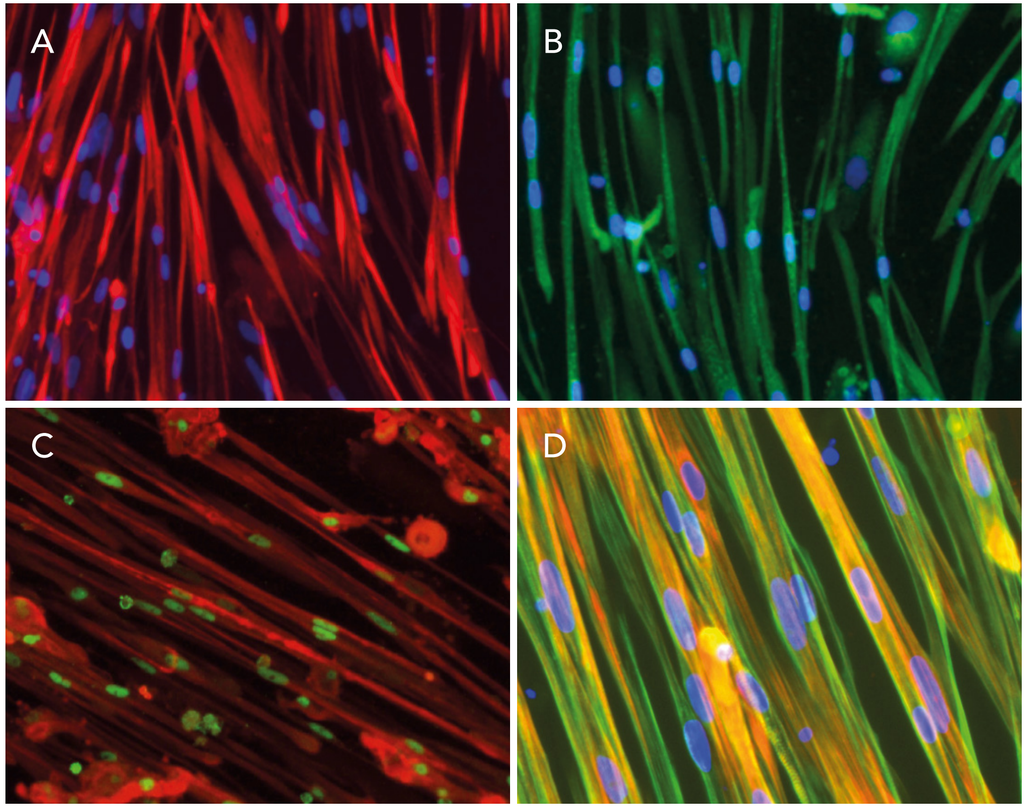
Skeletal myocytes express key myofilament proteins

Form visible striated fibres and multinucleation

Immunofluorescence staining at day 10 post-revival demonstrates robust expression of components of the contractile apparatus, including Desmin (A), Dystrophin (B), and Myosin Heavy Chain (C), along with the muscle transcription factor Myogenin (C). Cells also demonstrate expression of Troponin with visible striated fibres and multinucleation (D).

" An advantage of these cells is their higher population purity compared to other stem cell derived cells. This enables us to achieve higher numbers of functional striated muscle that are capable of contracting under electrical stimulation. This considerably increases the pace at which we can test our bioelectronics devices."

Amy Rochford, University of Cambridge



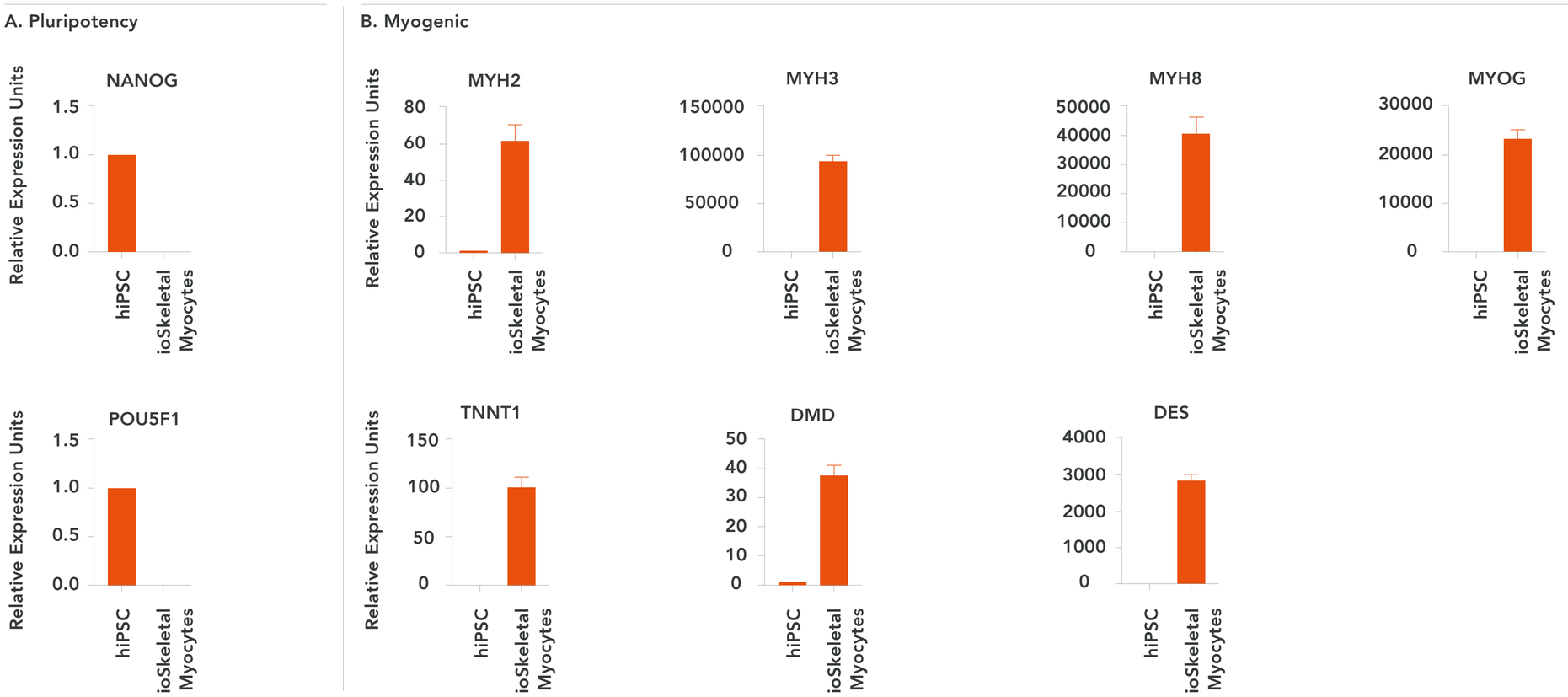
A. Desmin/DAPI
B. Dystrophin/DAPI
C. Myogenin/MHC
D. Troponin/Phalloidin/DAPI

Cells demonstrate gene expression of key myogenic markers by day 10 post-revival

Following reprogramming, ioSkeletal Myocytes downregulate expression of pluripotency genes (A), whilst demonstrating robust expression of key myogenic markers (B)

Gene expression levels assessed by RT-qPCR (data expressed relative to the parental hiPSC, normalised to HMBS).

Data represents Day 10 post-revival samples; n=7 biological replicates.



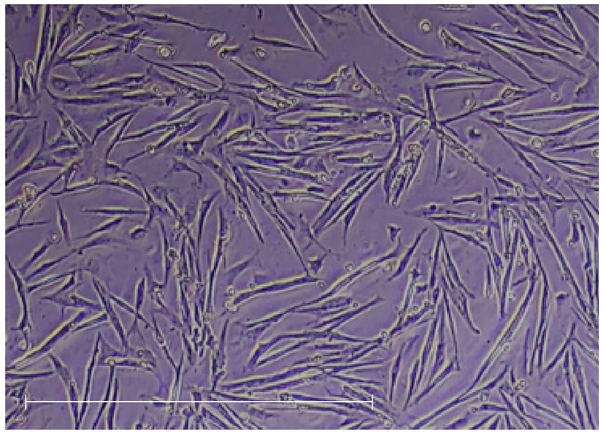
Classical myocyte morphology within days post-revival

Form elongated multinucleated myocytes over 10 days

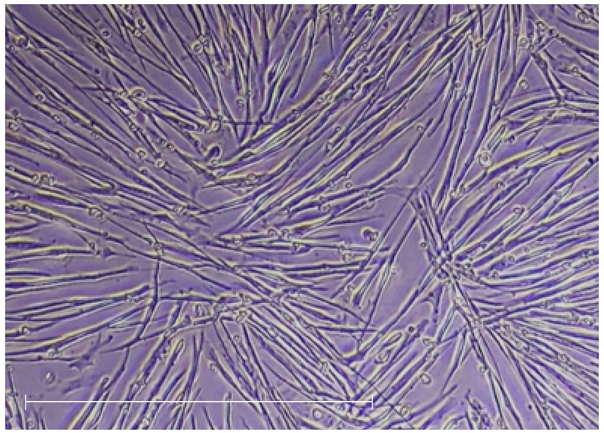
ioSkeletal Myocytes after revival over the course of the first 10 days of culture.

Day 3 to 10 post-thawing; 4X magnification; scale bar: 800µm.

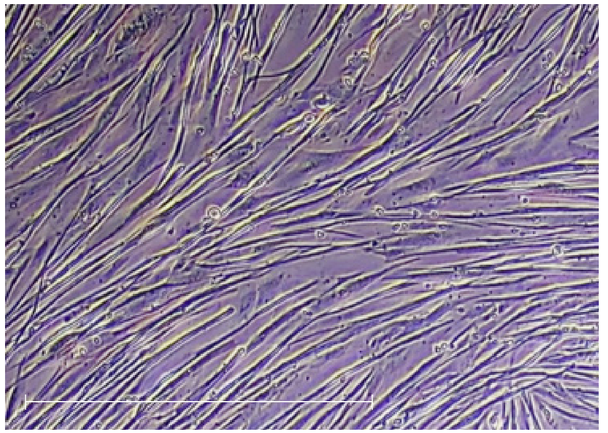
Day 3



Day 5



Day 10



Unique human muscle model for metabolic research

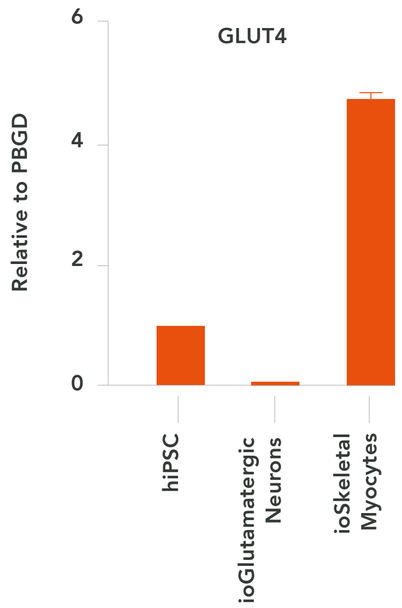
Myocytes express the insulin-regulated glucose transporter GLUT4

Critically for metabolic studies, data demonstrates expression of the insulin regulated glucose transporter GLUT4:

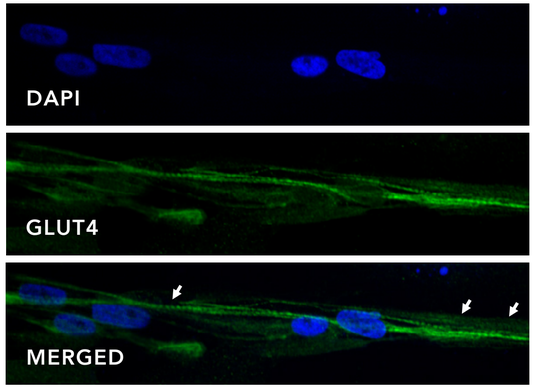
A. RT-qPCR at Day 10 post-revival demonstrating expression of GLUT4 in the ioSkeletal Myocytes, compared to undifferentiated hiPSCs and ioGlutamatergic Neurons.
B. Immunocytochemistry at Day 7 post-revival demonstrates expression of GLUT4 in peri-nuclear regions, and striations, in the ioSkeletal Myocytes*.
C. Western blotting of differentiated 3T3-L1 adipocytes and maturing ioSkeletal Myocytes demonstrates GLUT4 expression in a time-dependent manner*.

* Dougall Norris & Daniel Fazakerley,
Wellcome-MRC Institute of Metabolic Science

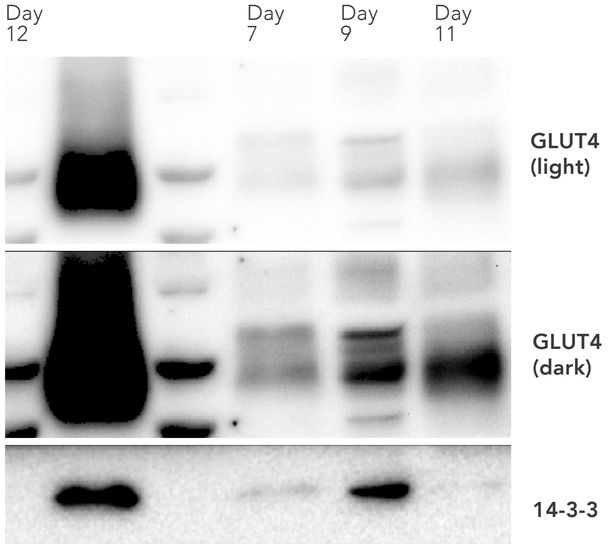
A. Gene expression



B. Immunocytochemistry*



C. Western blotting*



Contractile muscle cells for functional studies

Striated myocytes contract in response to electrical stimulation and increased extracellular potassium levels

Changes in intracellular Calcium (Ca^{2+}) content after chemical and electrical stimulation. Experiments performed at Day 10 post-revival.

A. Immunofluorescence staining of alpha-actinin in ioSkeletal Myocytes revealing robust expression of sarcomere structures.

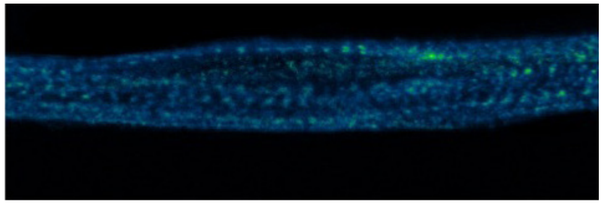
B. Representative images of ioSkeletal Myocytes incubated with Indo-1 AM 5 uM and 0.02% Pluronic F127. Cells were excited at 355nm on a UV-visible confocal, and emission measured simultaneously at 390 and 495nm.

C. Changes in Indo-1 AM ratio shows Ca^{2+} influx induced by 45 mM KCl.

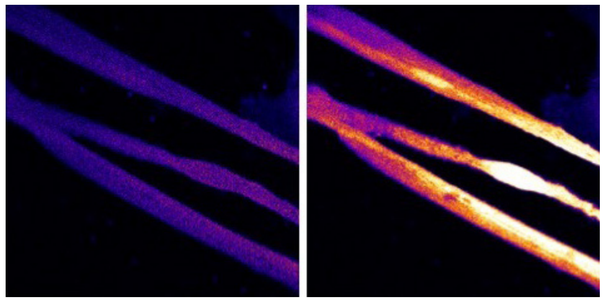
D–E. Electrical stimulation (2 Hz, 6 v, 2 ms) shows the repetitive ability of ioSkeletal Myocytes to induce calcium Ca^{2+} release and sequestration.

Gabriel E. Valdebenito & Michael R. Duchen, 2021.
Department of Cell and Developmental Biology and Consortium for Mitochondrial Research, UCL, London

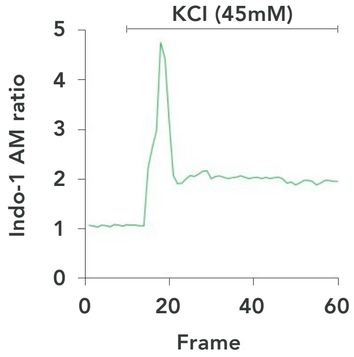
A



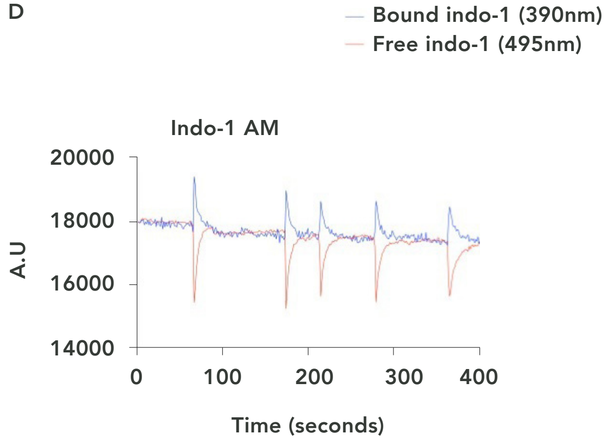
B



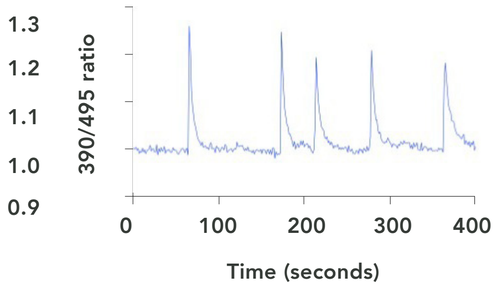
C



D



E



Robust and scalable cells suitable for phenotypic based high-throughput screening

ioSkeletal Myocytes generate myocytes within as little as 4 days post-revival with a high degree of MHC+ cells

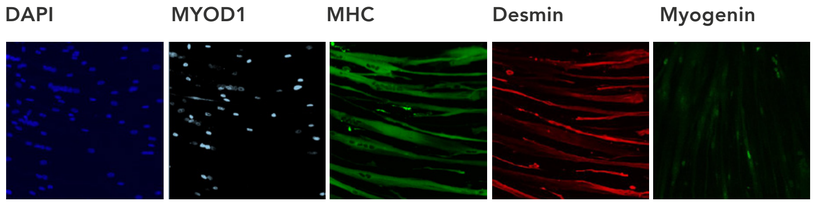
A. Human fibroblasts were transduced with lentiviral vectors allowing inducible over-expression of MYOD1 to transdifferentiate them to myocytes in approximately 10 days. Transdifferentiated myotubes were stained for multiple myotube markers to assess the purity and degree of multi-nucleation.

B. iPSCs stably and inducibly expressing MYOD1 using opti-ox technology (ioSkeletal Myocytes) can generate myocytes within as little as 4 days from revival with a high-degree of MHC+ cells (>95% purity), suitable for phenotypic based high throughput screens.

C. Comparable total area of MHC positive cells are generated between ioSkeletal Myocytes and transdifferentiated fibroblasts.

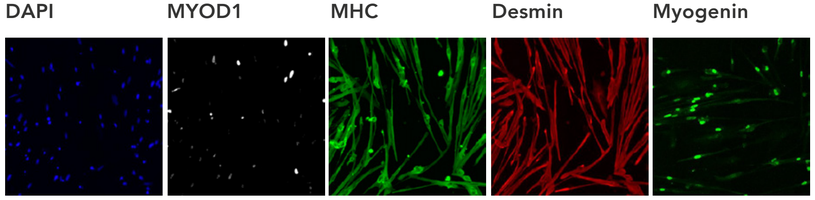
* Shushant Jain et al., Charles River Laboratories

A

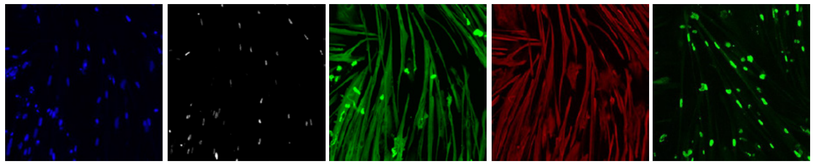


Day 10 transdifferentiated fibroblasts

B

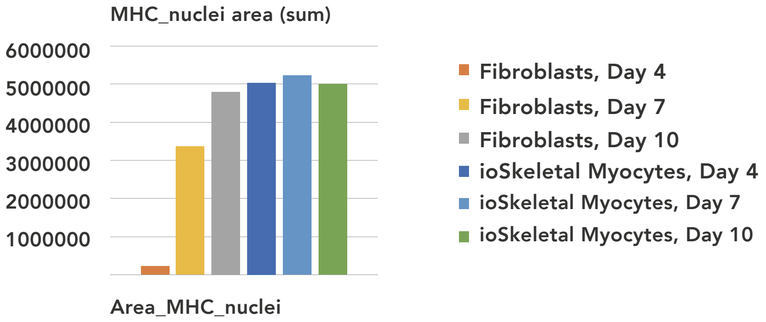


Day 4 ioSkeletal Myocytes



Day 10 ioSkeletal Myocytes

C



" One of the biggest advantages of the ioSkeletal Myocytes is within the early drug discovery phase. You can very quickly screen a large number of molecules in a short amount of time with minimal variability and high reproducibility."

Dr Shushant Jain,
Charles River

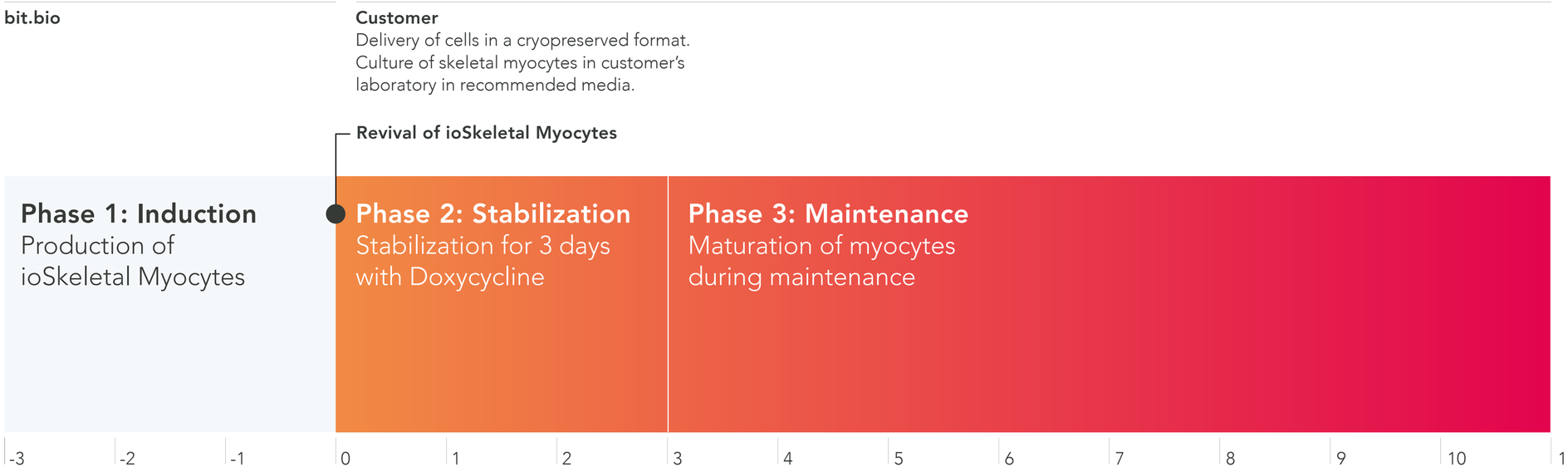
Ready-to-culture and easy-to-use

ioSkeletal Myocytes 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. Stabilisation for 3 days with Doxycycline
3. Maintenance during which the myocytes mature

> **" The ioSkeletal Myocytes have a much shorter cell culture time compared to harvesting primary muscle cells, saving us months on cell culture work."**
>
> Amy Rochford, University of Cambridge



Cost effective and flexible

ioSkeletal Myocytes 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 100,000 cells/cm^2.

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

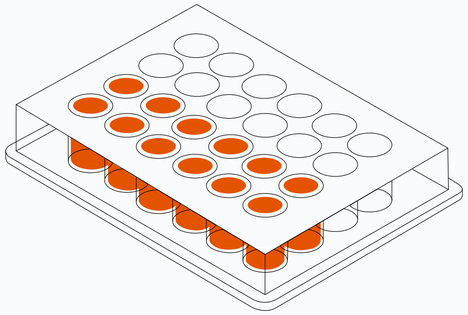
One Large vial can plate a minimum of 1 × 24-well plate, 1.5 × 96-well plate, or 2 × 384-well plates.

Recommended seeding density

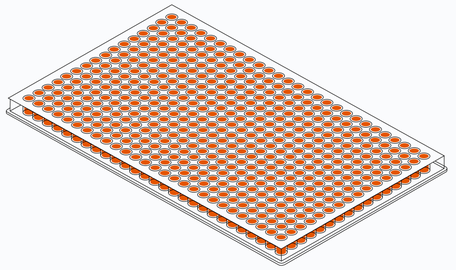
100,000 cells/cm^2

Small vial size

2.5 × 10^6 cells



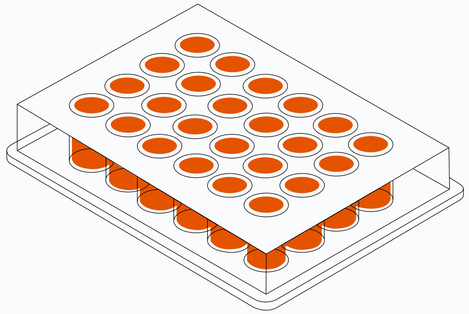
24-well plate



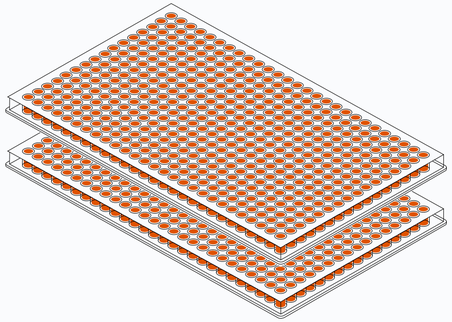
384-well plate

Large vial size

5 × 10^6 cells



24-well plate



384-well plate

Upcoming products

Our range is constantly expanding. Coming soon are iPSC-derived skeletal myocytes with disease-relevant mutations for modelling Duchenne Muscular Dystrophy.

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: >2.5 x 10^6 viable cells
Large: >5 x 10^6 viable cells

Recommended seeding density
100,000 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

- **Muscle, neuromuscular and metabolic research**
- **Drug development (e.g. high-throughput screening)**
- **Disease modelling**
- **Genetic screening (e.g. CRISPR Screening)**

Validated techniques include
- Contractility assays
- Co-culture
- Immunocytochemistry (ICC)
- Calcium signaling assays
- Single nuclei RNA sequencing (snRNA-seq)
- 3D cell culture

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