ioSkeletal Myocytes

Frequently Asked Questions

ioSkeletal Myocytes
Early Access Product
Catalogue no: EA1200

Frequently Asked Questions
Doc no: NPI-0002-FAQ V-01

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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 (≥2.5x10^6 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 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 ioSkeletal Myocytes cells fully differentiated?**
   No, ioSkeletal Myocytes are not fully differentiated when the end user receives them. ioSkeletal Myocytes are shipped as 'primed' skeletal myocytes 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 3 days with Doxycycline;
   3. Maintenance during which the skeletal myocytes mature (Figure 1).

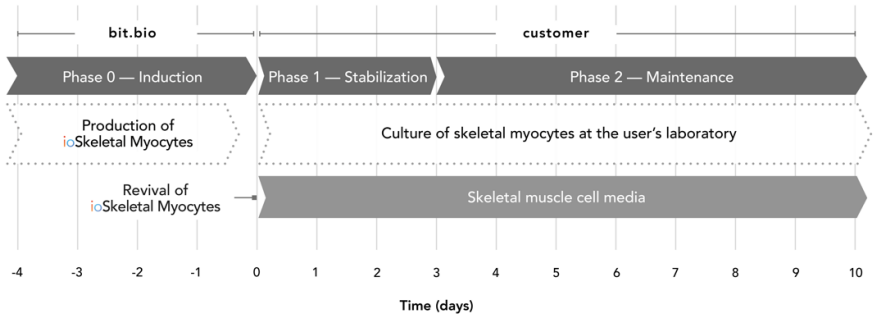

Figure 1
Schematic representation of the three-phase protocol to produce and culture ioSkeletal Myocytes.

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

3. **What seeding density do you recommend for the ioSkeletal Myocytes?**
   Skeletal myocyte cultures are obtained by plating ioSkeletal Myocytes at a minimum seeding density of 100,000 cells/cm^2; this may require optimisation depending on the experiment and plate format. bit.bio do not advise end users to seed below 100,000 cells/cm^2.

4. **How are cells cultivated?**
   Cells are cultivated in serum-free, chemically defined culture conditions, as 2-D mono-layer on Geltrex coated TC dishes (detailed composition can be found in the User Manual). They are cryopreserved in KnockOut serum replacement (CTS-grade) supplemented with 10% DMSO.

5. **How soon after delivery can ioSkeletal Myocytes be used for experiments?**
   ioSkeletal Myocytes are delivered in a cryopreserved format and are programmed to rapidly mature upon revival in the recommended media. By Day 3 post-revival, ioSkeletal Myocytes demonstrate classical myocyte morphology and express the myocyte genes Desmin, Myogenin, and Myosin Heavy Chain, as assessed by qRT-PCR. By Day 7 post-revival, skeletal myocytes demonstrate expression of GLUT4 in peri-nuclear regions and striations. Skeletal myocytes express the major proteins of myofilaments, including Myosin heavy chain, Desmin, Dystrophin and Troponin, and form striated multinucleated myocytes that contract in response to acetylcholine by Day 10 post revival.

Product information & quality control

1. **Why is opti-ox better than other methods of cellular reprogramming?**
   bit.bio ioSkeletal Myocytes are derived from human 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 reproducibility, homogeneity of differentiation and scalability compared to classical approaches using non-targeted transgenesis (e.g. viral vectors). ioSkeletal Myocytes are easy to culture and within days of revival convert into homogeneous and mature skeletal myocytes.

2. **What were the cells of origin for ioSkeletal Myocytes?**
   ioSkeletal Myocytes 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.

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4. **Do you use viral vectors to manufacture ioSkeletal Myocytes?**
   No, only recombinant DNA vectors are used to generate ioSkeletal Myocytes 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 ioSkeletal Myocytes is MYOD1. 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. **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 ioSkeletal Myocytes 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 High Glucose	Sigma	D6546	2°C to 8°C
Insulin-Transferrin-Selenium (ITS-G)	ThermoFisher	4140045	2°C to 8°C
Glutamax	ThermoFisher	35050061	2°C to 8°C
FGF2	qkine	Qk002	-20°C to -80°C
Retinoic Acid	Sigma	R2625	-20°C to -80°C
CHIR99021	Tocris	4423	-20°C to -80°C
Doxycycline	Sigma	D9891	2°C to 8°C
Bovine Serum Albumin	R&D Systems	RB02	2°C to 8°C

7. **What quality control is performed on the ioSkeletal Myocytes?**
   ioSkeletal Myocytes production batches are tested for sterility, viability and maturity acquisition over time by monitoring the expression of key genes by RT-qPCR: a loss of pluripotency (OCT4 and NANOG), and acquisition of Myosin Heavy Chains (MYH2, MYH3, MYH8), Troponin (TNNT1), along with Desmin (DES), Dystrophin (DMD) and the skeletal myocyte transcription factor Myogenin (MYOG). In addition, production batches are tested by immunocytochemistry for the expression of Myogenin, Desmin, Dystrophin and Myosin Heavy Chain. The genetic integrity of the parental hiPSC clone used for ioSkeletal Myocytes manufacturing is tested by G-banding karyotyping and array genome hybridisation (AGH). Absence of common human pathogens (Hepatitis B, Hepatitis C, HIV1, HIV2, HTLV 1, HTLV 2), mycoplasma, bacterial and fungal growth is confirmed by validated means.

8. **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.

9. **Can you please provide some references for ioSkeletal Myocytes cells?**
   Please refer to the publication describing the reprogramming of human iPSCs into skeletal myocytes by MYOD driven opti-ox cellular reprogramming:
   - Pawlowski, M. et al. 2017. Inducible and Deterministic Forward Programming of Human Pluripotent Stem Cells into Neurons, Skeletal Myocytes, and Oligodendrocytes. Stem Cell Reports 8(4): 803-812.

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