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ab277612 ioSkeletal Myocytes– Human iPSC-Derived Skeletal Myocytes

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For conversion into consistent, striated and multinucleated skeletal myocytes, providing a high quality model for the study of muscle, the neuromuscular junction, and associated metabolic disorders.

In partnership with bit.bio

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ioSkeletal Myocytes are delivered in a convenient cryopreserved format and rapidly mature into functional skeletal myocytes after revival in the recommended medium. Skeletal myocytes are ready for experiments as soon as day 2 post-revival and contractility can be assessed as early as 3 days post-revival. Cells are kept in a single culture medium formulation from revival with fully disclosed composition allowing modifications to fit customer's bespoke experiments.

ioSkeletal myocytes cultures display classical myocyte morphology, forming elongated multinucleated myocytes over 10 days. The protocol for the generation of ioSkeletal Myocytes is a three-phase process. Phase 0 - Induction is carried out at bit.bio before distribution (Fig 1).

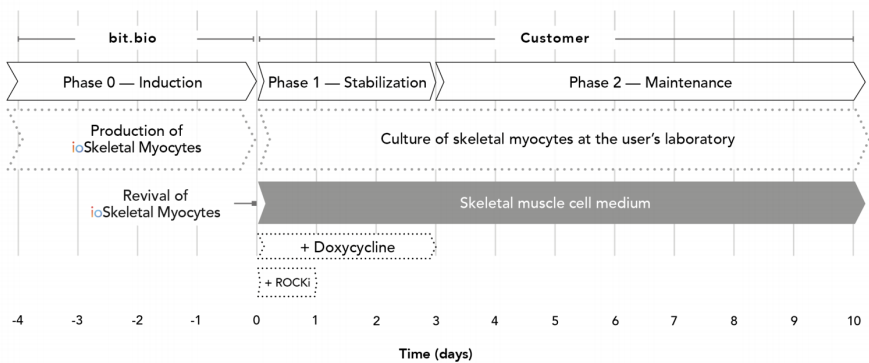


Figure 1 Schematic representation of the three-phase protocol to produce and culture ioSkeletal Myocytes.

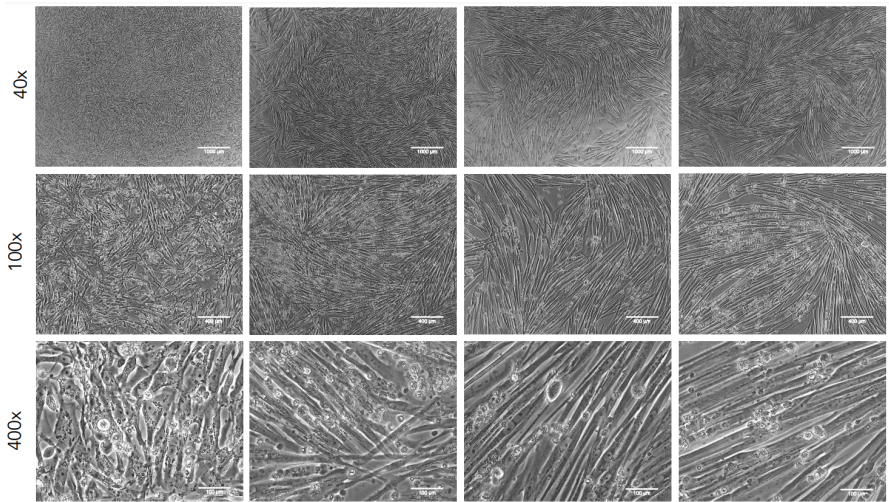
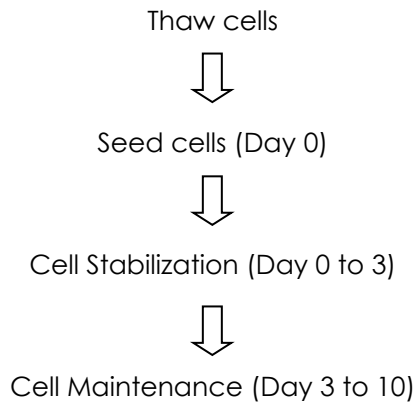


Figure 2 Photos of skeletal myocytes after revival at the user's laboratory over the course of the first 10 days of culture (Day 1 to 10 post-thawing; 40X magnification; scale bar: 1000 μ m, 100X magnification; scale bar: 400 μ m, 400X magnification; scale bar: 100 μ m).

	<p>a 4-day induction protocol. This leads to an irreversible loss of pluripotency, and the synchronised homogenous production of skeletal myocytes. The ioSkeletal Myocytes are cryopreserved as single cells prior to distribution.</p>
User	<p>Phase 1 — Stabilization (day 0 to 3): The ioSkeletal Myocytes are revived at the user's laboratory using the recommended medium supplemented with ROCKi (24h) for enhanced post-thawing viability and Doxycycline (72h) for sustained induction.</p>
	<p>Phase 2 — Maintenance (day 3 to 10): After the 3-day stabilization, the culture shows homogenous skeletal myocyte phenotype based on cell morphology and protein expression of myosin heavy chain (MHC), desmin and titin. The cells can be used for a variety of biological assays and depending on assay requirements, ioSkeletal Myocytes can be used over different lengths of time in the maintenance medium (typically for up to 8 days*).</p> <p>*Note: the mature myocytes tend to detach over time in culture while acquiring spontaneous contractibility. This is more marked from low density cultures and will vary depending on the culture matrix used.</p>

Table 1 Description of the three-phase protocol for the production and culture of ioSkeletal Myocytes.

2. Protocol Summary



3. Materials Supplied and Storage

Transfer the vials of ioSkeletal Myocytes to liquid nitrogen or to -150°C immediately after receipt.

Before the revival of ioSkeletal Myocytes, prepare the tissue culture plates or flasks coated with Geltrex (refer to Section 6.3)

The recommended reagents for the revival and maintenance of ioSkeletal Myocytes can be found in Section 4.

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully culture the cells:

- Biological Safety Cabinet
- Normoxic cell culture incubator (37°C, 5% CO₂)
- 37°C water bath or equivalent
- Haemocytometer or automatic cell counter
- Liquid Nitrogen Storage Unit
- Standard tissue culture wares (pipettes, tips, culture plates)
- Bench Top Centrifuge
- ROCKi (Y-27632) (ab120129)
- Geltrex (Reduced GF)
- DMEM/F-12
- Insulin-Transferrin-Selenium (ITS-G) (100X)
- N-2 Supplement (100X)
- Glutamax (100X)
- Knockout Serum Replacement (KOSR)
- Doxycycline (ab141091)

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the cells, please see the ioSkeletal Myocytes cells datasheet on our website.

6. Reagent Preparation

6.1 Preparation of stock solutions

Reagent	Stock Solution	Working concentration
ROCKi (Y-23632) (ab120129)	10 mM (1,000X solution) To prepare, reconstitute 10 mg in 3037 μ L of PBS	10 μ M 1 μ L of stock solution per 1 mL of medium
Doxycycline (DOX) (ab141091)	2 mg/mL (2,000X solution) To prepare, reconstitute 20 mg in 10 mL of H ₂ O	1 μ g/mL 0.5 μ L of stock solution per 1 mL of medium

6.2 Preparation of Skeletal Myocytes Medium

b:SM: Skeletal Myocytes Basal Medium

Reagent/Media	For 200 mL	For 500 mL
DMEM (High Glucose)	196 mL	490 mL
Glutamax (100X)	2 mL	5 mL
ITS-G (100X)	2 mL	5 mL

Δ Note: The basal medium is stable for 3 weeks at 4°C; Pen/Strep antibiotics can be added if required.

comp:SM: Skeletal Myocytes Complete Medium

Reagent/Media	For 50 mL	For 200 mL
b:SM	44.5 mL	178 mL
KOSR	5 mL	20 mL

N-2 Supplement (100X)	500 μ L	2 mL
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Δ Note: The complete medium stable for 2 weeks when stored at 2°C to 8°C protected from light.

comp:SM+R+D: Supplemented Skeletal Myocytes Complete Medium

Reagent/Media	For 10 mL	For 50 mL
comp:SM	10 mL	50 mL
ROCKi (final conc. 10 μ M)	10 μ L	50 μ L
Doxycycline (final conc. 1 μ g/mL)	5 μ L	25 μ L

Δ Note: The supplemented complete medium is better prepared fresh before each feeding. It is not recommended to use the supplemented complete medium for more than 4 days after preparation while stored at 4°C.

6.3 Preparation of the Geltrex coating solution and coated vessels

For the preparation of Geltrex aliquots and Geltrex coated plates, please follow the manufacturer's instructions. In brief:

6.3.1 Preparation of Geltrex aliquots:

6.3.1.1 Remove Geltrex stock aliquots from -80°C and thaw once in a 4°C fridge overnight. The next day, prepare aliquots according to foreseen use in order to minimize further freeze-thawing; store at -80°C.

Δ Note: Geltrex solidifies quickly at temperatures above 4°C — keep Geltrex on ice at all times.

6.3.1.2 Depending on the volume, the smaller aliquots should take about 30 minutes to thaw on ice.

6.3.2 Geltrex coating

6.3.2.1 Calculate the total surface area to be coated.

6.3.2.2 Dilute the Geltrex 1:100 in chilled DMEM/F12 (e.g. 100 μ L in 10 mL)

6.3.2.3 Coat the surface area of your culture vessel with the Geltrex:DMEM coating solution.

6.3.2.4 We recommend the follow coating volumes (circa 100 μ L per cm²)

Coating solution	96 well	12 well	6 well	T25 flask	10 cm dish
Geltrex:DMEM/F1 2	50 μ L	500 μ L	1 mL	3 mL	6 mL

6.3.2.5 Incubate the coated plates at 37°C for a minimum of 60 minutes. At the time of use, we recommend keeping the plates at room temperature for one hour before aspirating. Carefully aspirate off the excess Geltrex, then immediately plate the cells.

Δ Note: Geltrex coated plates can be kept at 4°C for 1 month if sealed with parafilm.

7. Assay Procedure

7.1 Culture of ioSkeletal Myocytes:

7.1.1 Before starting:

- Each ioSkeletal Myocytes vial contains $< 2.5 \times 10^6$ (Small vial) or $> 2.5 \times 10^6$ (Large vial) cells. A seeding density of 100,000 cells/cm² is recommended. Prepare enough tissue culture vessels with Geltrex coating prior to reviving the cryovial(s) (see Section 6.3).
- Warm-up the water bath to 37°C.
- Warm-up basal skeletal myocytes medium (b:SM) medium to 37°C (see Section 6.2).
- Prepare the complete skeletal myocytes (comp:SM) medium supplemented by 10 μ M ROCKi and 1 μ g/mL Doxycycline (comp:SM+R+D) for revival (see Section 6.2).

7.1.2 Cell Thawing:

- 7.1.2.1 Remove the cryovial(s) from dry ice and immediately immerse into a 37°C waterbath (or similar) while maintaining a constant gentle agitation.
- 7.1.2.2 Remove the cryovial(s) from the water bath when only a very small ice cube is left visible (this should take approximately 1 minute).
- 7.1.2.3 Spray the cryovial(s) with 70% ethanol and take it to a biological safety cabinet.
- 7.1.2.4 Transfer the cells from each vial into a 15 mL tube containing 1 mL of b:SM medium.
Δ Note: Freezing medium contains DMSO: minimize the time between thawing and centrifugation of cells.
- 7.1.2.5 Add a further 3 mL of b:SM medium per tube in a dropwise manner.
- 7.1.2.6 Carefully wash the cryovial(s) with 1 mL of b:SM medium and add it to the tube(s).
- 7.1.2.7 Centrifuge the cells at 300 x *g* for 3 minutes at room temperature.
- 7.1.2.8 Carefully remove the supernatant by aspiration.
- 7.1.2.9 Add 3 mL of comp:SM+R+D medium to the cell pellet and gently resuspend the cells by pipetting up-and-down with a 1 mL micropipette.

7.1.2.10 Count the cells including a cell viability marker. The typical recovery from one cryovial is $>2.5 \times 10^6$ viable cells (Small vial) and $>5 \times 10^6$ viable cells (Large vial).

7.1.3 Cell Seeding (Day 0):

7.1.3.1 Dilute the cell suspension to the required cell concentration using comp:SM+R+D medium to achieve the required seeding density for your desired experimental conditions. A seeding density of 100,000 cells/cm² is routinely used.

Plate format	Surface (cm ²)	mL/well	Cells/well	Cells/mL
6 well	9.5	2.5	950,000	380,000
12 well	3.8	1	380,000	380,000
24 well	1.9	0.5	190,000	380,000
48 well	0.95	0.25	95,000	380,000
96 well	0.32	0.1	32,000	320,000
384 well	0.056	0.03	5,600	187,000

7.1.3.2 Aspirate the Geltrex coating solution from the culture vessel(s).

7.1.3.3 Directly add the required volume of cell suspension to the culture vessel(s).

7.1.3.4 Immediately transfer the culture vessel(s) to a standard normoxic tissue culture humidified incubator at 37°C, 5% CO₂.

7.1.3.5 To ensure an even cell distribution, gently cross-shake the plate once on the incubator shelf (back and forth, side to side, 2-3 times).

7.1.4 Cell Stabilization (Day 0 to 3) and Maintenance (Day 3 to 10):

7.1.4.1 Day 1: 48 hours post thawing, completely replace the culture medium with fresh pre-warmed comp:SM medium supplemented with 1 µg/mL doxycycline (no ROCKi).

7.1.4.2 Day 2: 48 hours post thawing, completely replace the culture medium with fresh pre-warmed comp:SM medium supplemented with 1 µg/mL doxycycline (no ROCKi).

- 7.1.4.3 Day 3: 72 hours post thawing, completely replace the culture medium with fresh pre-warmed comp:SM medium (no doxycycline/no ROCKi).
- 7.1.4.4 Day 5-10: for optimal skeletal myocytes maintenance, it is recommended a complete medium change every 48 hours with fresh comp:SM medium (no Doxycycline/no ROCKi).

8. Typical Data

Data provided for demonstration purposes only.

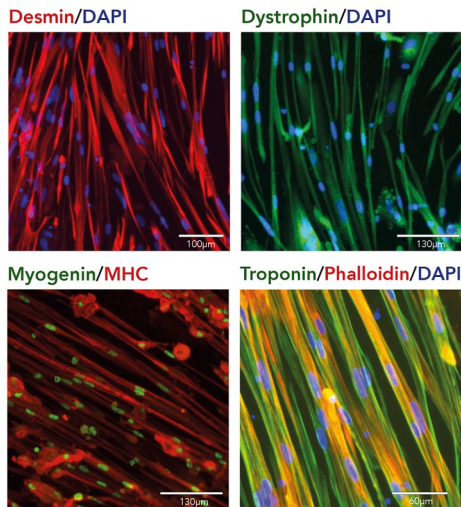


Figure 3. Immunofluorescence staining at day 10 post revival demonstrates robust expression of components of the contractile apparatus such as Desmin (top left), Dystrophin (top right), and Myosin Heavy Chain (bottom left), along with the muscle transcription factor Myogenin (bottom left). Cells also demonstrate expression of Troponin with visible striated fibres and multinucleation (bottom right).

9. Notes

Technical Support

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