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# Human Skeletal Muscle Myoblasts (iPSC-derived)

Catalog Number	40HU-176 (Normal) 40HU-178 (ALS)	Cell Number	1 million cells/vial (Cryopreserved) 3 million cells/vial (Cryopreserved)	
Species	Homo sapiens	Storage Temperature	Liquid nitrogen	

## **Product Description**

iPSC-derived skeletal muscle myoblasts are valuable tools for biochemical analysis, disease modelling.

**iXCells Biotechnologies** is proud to provide ready-to-use human iPSC-derived skeletal muscle myoblasts for differentiation into functional myotubes. iXCells<sup>™</sup> hiPSC-derived myoblasts express typical markers, e.g. MyoD and Desmin (Figure 1) and rapidly differentiate into functional myotubes expressing markers including MHC, Dystrophin and MyoG (Figure 2), with the purity higher than 85%. Functional validation of iPSC-derived myotubes can be observed by their spontaneous twitching in the well.

iXCells<sup>™</sup> human iPSC-derived skeletal muscle myoblasts are available as a cryopreserved product (1 or 3 million cells/vial) and can be purchased in conjunction with the necessary media components (Cat# MD-0102A and Cat# MD-0102B) to expand, differentiate and maintain the myotubes in culture. Myotubes can be cultured for up to 4 weeks under special conditions.



**Figure 1.** MyoD (green) and Desmin (red) immunostaining of human iPSC-derived myoblasts 3 Days after recovery in **Myoblast Expansion Medium** (Cat# MD-0102A).

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**Figure 2.** Formation of myotubes is observed after 4 days of differentiation of human iPSC-derived myoblasts in **Myoblast Differentiation Medium** (Cat# MD-0102B). MHC staining of myotubes is shown by immunofluorescence staining (green).

## **Product Details**

Tissue Origin	Human iPSC-derived myoblasts (Normal, ALS)		
Package Size	1.0 million cells/vial (Frozen) 3.0 million cells/vial (Frozen)		
Shipped	Cryopreserved		
Storage	Liquid Nitrogen		
Media	iPSC-Derived Myoblast Expansion Medium (Cat# MD-0102A) iPSC-Derived Myoblast Differentiation Medium (Cat# MD-0102B)		

## **Protocols**

### Mono-culture of hiPSC-Derived Myoblasts

### The following protocol is based on multi-well plate format

- 1. Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture as soon as possible in order to retain the highest cell viability.
- 2. Prepare Collagen I-coated plates the day before seeding of myoblasts. Add the amount of Collagen I (Corning, Cat# 354236) into 0.01 N HCI (diluted in DPBS) as described in the table below. Incubate vessel at 37°C for 2 hours, or overnight at room temperature. Following incubation, aspirate the Collagen I solution from the coated surface, remove vessel lid and allow to dry at least 2 hours in a Biosafety Cabinet. Once dried, wash the culture vessel 3X with sterile tissue culture grade water. The coated plates can be used immediately, or stored (dried) at 4°C for up to 2 weeks.

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Vessel name	Collagen I (µg/well)	Volume (mL)
96-well plate	10	0.1
48-well plate	20	0.2
6-well plate	100	1
10cm Plate	500	5

- 3. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the risk of contamination.
- 4. Pipette the cells into a 15 mL conical tube with 5 mL Myoblast Expansion Medium (Cat# MD-0102A).
- 5. Centrifuge at 200*g* for 5 minutes at room temperature.
- 6. Remove the supernatant and re-suspend the cells in **Myoblast Expansion Medium**.
- 7. Seed the cells on Collagen I-coated plates at the desired density.

*Note:* We recommend seeding the myoblasts at 15,000 – 40,000 cells / cm<sup>2</sup>, lower densities will take longer to reach the 80% confluence required for differentiation

- 8. Incubate in 37°C CO<sub>2</sub> incubator overnight.
- 9. Perform medium change every 2 days until the cells reach ~80-90% confluence.
- 10. Replace media with **Myoblast Differentiation Medium** (Cat# MD-0102B) and incubate for 4-5 days to allow for complete differentiation. Cell morphology will dramatically change with cells becoming elongated and aligned within the well.

### **Disclaimers**

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