www.ixcellsbiotech.com
10340 Camino Santa Fe, Suite C, San Diego, CA 92121
Tel: (858)412-5988 Fax: (858)368-8716
Technical Supports: supports@ixcellsbiotech.com
Orders: orders@ixcellsbiotech.com

Product Information

iXCells™ Human iPSC-Derived Myoblasts

Catalog Number	40HU-176 40HU-176D	Cell Number	1 million cells/vial (Cryopreserved) 3 million cells/vial (Cryopreserved)
Species	*Homo sapiens*	Storage Temperature	Liquid nitrogen, Media at -20C

Product Description

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

iXCells Biotechnologies is proud to provide commercial human iPSC-derived myoblasts for differentiation into functional myotubes. iXCells™ 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™ myoblasts are available in a kit format containing both cryopreserved vials (1 or 3 million cells/vial) along with the necessary media components to expand, differentiate and maintain the myotubes in culture for at least 4-6 weeks. Longevity of the myotubes in culture can be extended with a bi-weekly 24-hr pulse of Myoblast Expansion Medium followed by replacement with Myoblast Differentiation Medium.

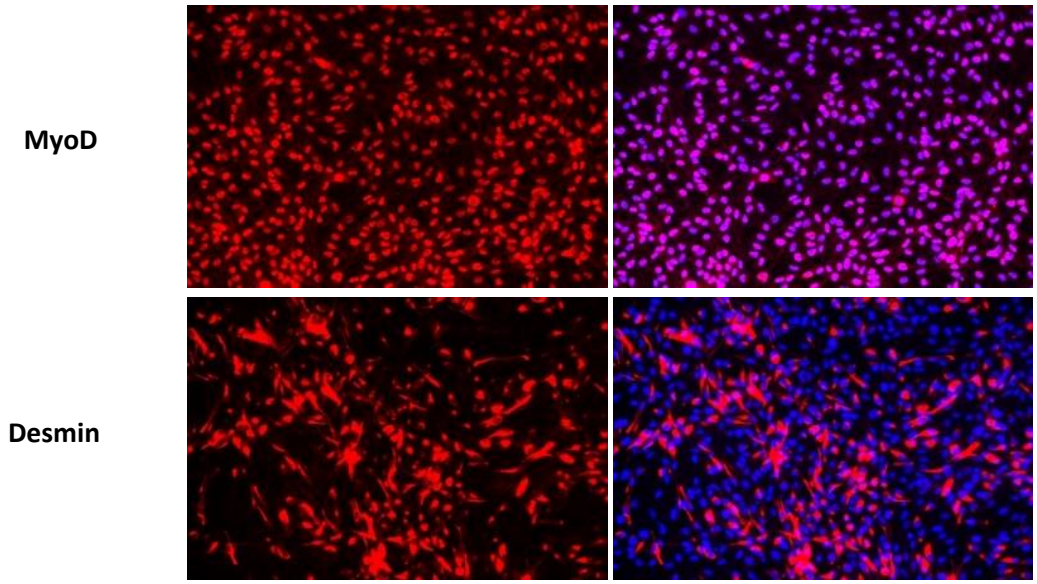

Figure 1. MyoD and Desmin staining of iPSC-derived myoblasts upon recovery in Myoblast Expansion Medium.

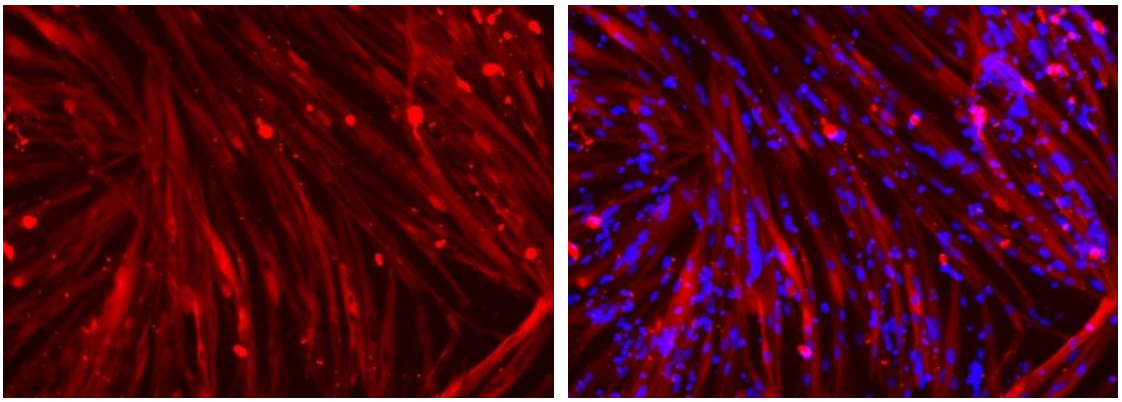

Figure 2. Formation of myotubes were observed after 4 days of differentiation of iPSC-derived myoblasts in Myoblast Differentiation Medium. MHC staining of myotubes were shown by immunofluorescence staining (red).

Product Details

Tissue Origin	Human iPSC-derived myoblasts (Normal, ALS)
Kit Contents	Complete kit containing both cryopreserved cells along with expansion and differentiation media
Cryopreserved Cell contents	1.0 million cells/vial (Frozen) 3.0 million cells/vial (Frozen)
Shipped	Cryopreserved
Media	**Myoblast Expansion Medium** (Catalog#: MD-0102A) **Myoblast Differentiation Medium** (Catalog#: 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. Add the amount of Collagen I (Corning, Cat.# 354236; 3.9mg/ml) into 0.01 N HCl as described in the table below. Add Collagen I solution with the suggested volume onto the culture vessels and incubate at 37°C for 2 hours or at RT for overnight. Remove the Collagen I solution from the coated surface. Remove the lid and allow it to air dry overnight in a Biosafety Cabinet. Rinse the culture vessels with sterile tissue culture grade water or PBS before introducing cells

and medium. It is not necessary to air dry the culture vessels. The coated plates can be stored at refrigerator up to 2 weeks.

Vessel name	Growth area	Collagen I (ug/well)	0.01N HCl (ml)	3.9mg/ml Collagen I/well (ul)
96-well plate	0.32 cm^2	3.2	0.02	1
48-well plate	0.95 cm^2	9.5	0.1	2.5
6-well plate	9.5 cm^2	95	1	25
T75 flask	75 cm^2	750	5	200

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 15ml conical tube with 5ml **Myoblast Expansion Medium (Catalog#: MD-0102A)**.
5. Centrifuge at 200x 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^2, lower densities will take longer to reach the 80% confluence required for differentiation
8. Incubate in 37°C CO_2 incubator overnight.
9. Perform medium change every 2 days until the cells reach ~80-90% confluence.
10. Replace media with **Myoblast Differentiation Medium** (Catalog#: 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.
11. Prior to initiation of downstream applications remove **Myoblast Differentiation Medium** and perform an overnight "pulse" of the cells with **Myoblast Expansion Medium.**
12. Replace media with **Myoblast Differentiation Medium** and execute downstream applications, change media accordingly in culture.

Co-culture of hiPSC-Derived Myotubes with Motor Neurons

The following protocol is based on 96-well plate format.

1. Follow protocol for mono-culture of iPSC derived myoblasts to generate mature myotubes in well through Step #11.
2. Thaw a vial of iXCells™ hiPSC-derived motor neurons (**Catalog#: 40HU-005**).
3. Remove **Myoblast Differentiation Medium** and seed motor neurons on top of myotubes in **Motor Neuron Maintenance Medium (Cat# MD-0022)**. It is recommended to seed 5,000-20,000 motor neurons in each well of a 96-well plate.
4. Muscle contractions can be observed as early as 5 days after co-culturing with motor neurons.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While iXCells Biotechnologies uses reasonable efforts to include accurate and up-to-date information on this product sheet, we make no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. iXCells Biotechnologies does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. iXCells Biotechnologies is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of strains on deposit, iXCells Biotechnologies is not liable for damages arising from the misidentification or misrepresentation of cultures.