

# MUSCLE CELL RESEARCH

Derive, Expand, and Differentiate Human Skeletal Muscle Progenitor Cells

## MyoCult™: A Complete Workflow

Skeletal muscle progenitor cells (also known as satellite cells) are studied to gain insight into muscle biology and are used for preclinical investigations into muscle-related diseases and drug discovery. The MyoCult™ Workflow supports your muscle research from start to finish, allowing you to derive, expand, and differentiate human skeletal muscle progenitors. A schematic presentation of the workflow is illustrated in Figure 1.

### MyoCult™-SF Expansion Supplement Kit (Human)

The serum-free MyoCult™-SF Expansion Supplement Kit (Human; Catalog #05980) is optimized to derive and expand PAX7<sup>+</sup> human skeletal muscle progenitors following tissue isolation (Figure 2). Complete MyoCult™-SF Expansion Medium is prepared by combining the expansion supplement with the basal DMEM medium (Catalog #36253). MyoCult™-expanded myoblasts generate a greater number of cells per passage compared to serum-containing media (Figure 3). Cryopreserved and human pluripotent stem cell (hPSC)-derived myoblasts can also be expanded using this kit (Figure 4).

### Why Use the MyoCult™-SF Expansion Supplement Kit (Human)?

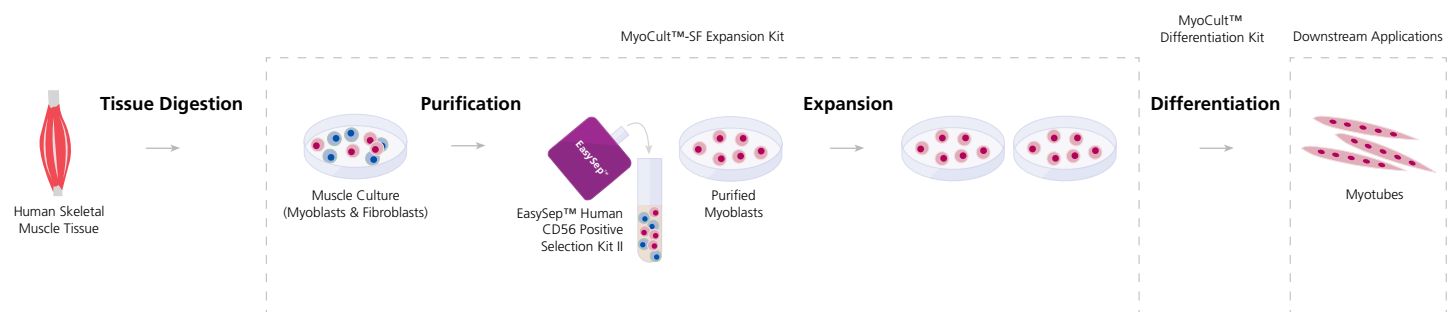
**CONSISTENT.** Serum-free formulation improves experimental reproducibility.

**HIGH PERFORMANCE.** Superior cell expansion when compared to serum-containing media.

**FUNCTIONAL.** Cultured myoblasts retain robust expansion and differentiation capacities.

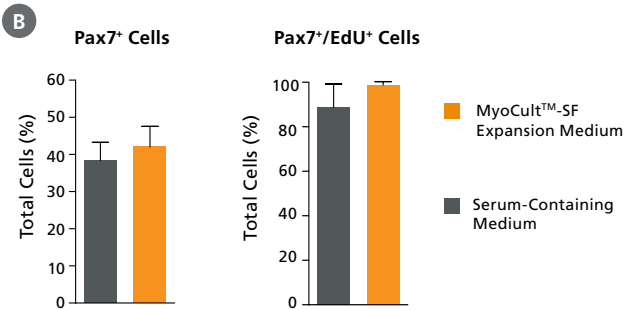
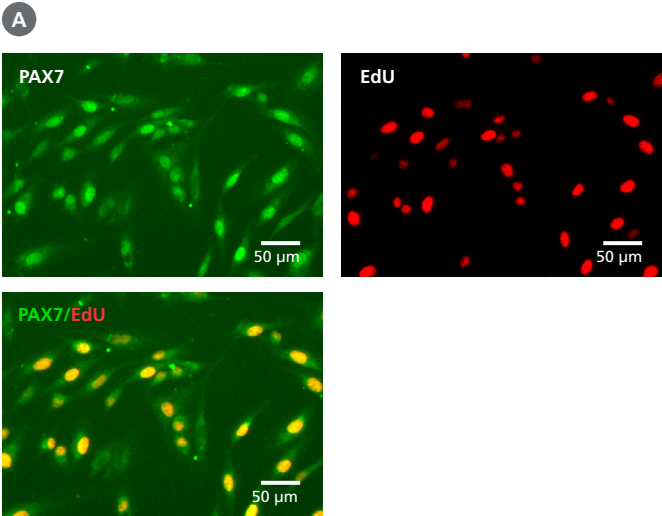
**OPTIMIZED.** Supports myoblast derivation and expansion directly from primary human skeletal muscle progenitors and cryopreserved or hPSC-derived myoblasts.

Primary skeletal muscle progenitors can be derived using MyoCult™-SF Expansion Medium and enriched further by the EasySep™ Human CD56 Positive Selection Kit II (Catalog #17855). The resulting cells, known as myoblasts, retain a robust differentiation capacity (Figures 4,5) and can be used for a wide range of downstream applications.



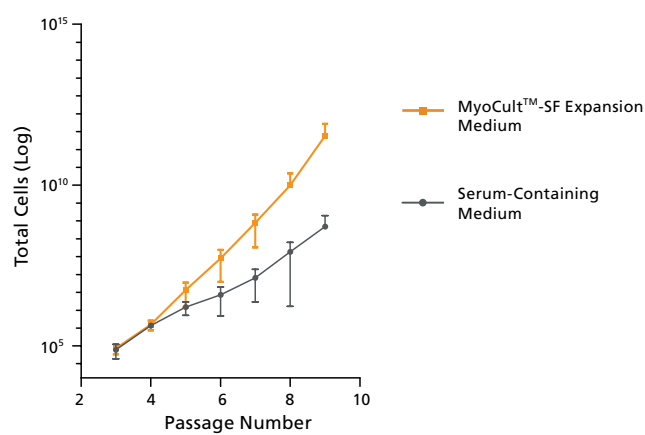
**Figure 1.** The MyoCult™ Workflow for Derivation, Expansion, and Differentiation of Human Skeletal Muscle Progenitor Cells

Human skeletal muscle tissue is digested into a single-cell suspension and plated into MyoCult™-SF Expansion Medium. Myoblasts are then enriched by cell sorting or using a selection kit such as the EasySep™ Human CD56 Positive Selection Kit II. Purified myoblasts can be expanded for downstream applications or differentiated into myotubes using the MyoCult™ Differentiation Kit (Human; Catalog #05965).



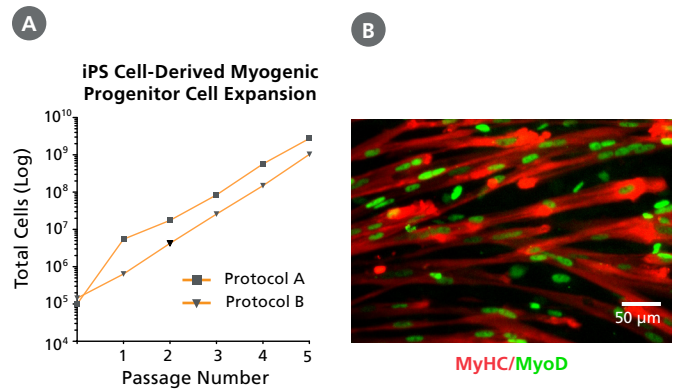
**Figure 2. MyoCult™-SF Expansion Medium Derives PAX7<sup>+</sup> Skeletal Muscle Progenitor Cells From Human Muscle**

Skeletal muscle progenitor cells were derived from human skeletal muscle tissue and expanded using the MyoCult™-SF Expansion Kit or a serum-containing medium. (A) Following 6 days of expansion, myoblasts were fixed and immunostained for PAX7 (green) and a thymine analogue (EdU; red) to identify proliferating myogenic progenitors. (B) Total percentage of cells expressing PAX7, or PAX7 and EdU were quantified (n = 4). Data were generated and used with permission by Dr. Penney M. Gilbert's Lab, Department of Biochemistry, University of Toronto. Error bars represent standard error of mean (SEM).



**Figure 3. MyoCult™-SF Expansion Medium Enables Long-Term Expansion of Primary Human Myoblasts**

Expansion of myoblasts in MyoCult™-SF Expansion Medium generated a greater yield of CD56<sup>+</sup> cells after 9 passages compared to myoblasts cultured in serum-containing medium (P9; n = 2). Data were generated and used with permission by Dr. Penney M. Gilbert's Lab, Department of Biochemistry, University of Toronto. Error bars represent SEM.

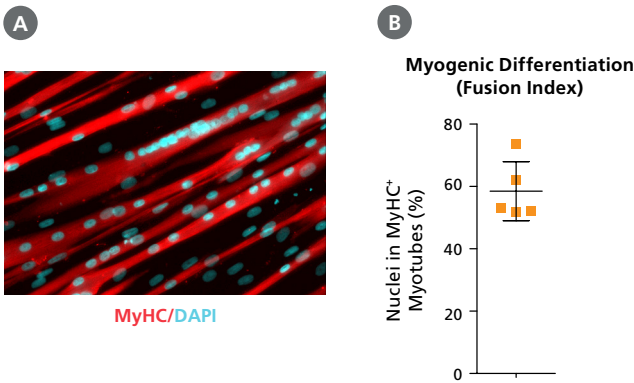


**Figure 4. MyoCult™-SF Expansion Medium Supports Expansion of Human iPS Cell-Derived Skeletal Muscle Progenitor Cells**

(A) Induced pluripotent stem (iPS) cell-derived skeletal progenitor cells displayed long-term expansion when using the MyoCult™-SF Expansion Supplement Kit (Human). iPS cell-derived skeletal progenitor cells were generated using the Chal et al. (Protocol A) and Xi et al. (Protocol B) methods.<sup>1-2</sup> (B) Culture-expanded iPS cell-derived skeletal progenitor cells were differentiated using the MyoCult™ Differentiation Kit (Human) and expressed MyHC (red) and MyoD (green). Similar results were observed for embryonic stem (ES) cell-derived muscle progenitors.

## MyoCult™ Differentiation Kit (Human)

The MyoCult™ Differentiation Kit (Human; Catalog #05965) is optimized for the in vitro differentiation of human myoblasts into multinucleated myotubes. The Animal Component-Free (ACF) Attachment Substrate included in this kit enhances differentiation and supports myotube attachment to cultureware. Myoblasts treated with the MyoCult™ Differentiation Kit (Human) can differentiate into fully matured myotubes with a high fusion index in as little as five days (Figure 5).



**Figure 5.** Primary Human Myoblasts Expanded in MyoCult™-SF Expansion Medium Maintain Differentiation Capacity

Myoblasts derived and expanded in MyoCult™-SF Expansion Medium were further differentiated into myotubes using the MyoCult™ Differentiation Kit (Human) at passage 5. (A) Myotubes differentiated from myoblasts were immunostained for myosin heavy chain (MyHC; red) and nuclei (DAPI; blue). (B) Approximately 60% of total nuclei were associated with MyHC-expressing myotubes, indicative of high fusion indices of MyoCult™-expanded myoblasts. Each donor is indicated by an orange square. Data are from 5 independent donors. Error bar represents SEM.

### Why Use the MyoCult™ Differentiation Kit (Human)?

- RELIABLE.** Rigorous raw material screening and quality control minimize experimental variability and increase reproducibility.
- EFFICIENT.** Fast and robust differentiation of human myoblasts into fully mature myotubes.
- OPTIMIZED.** Attachment substrate supports cell adherence to cultureware and enhances myotube morphology.

## Product Information

PRODUCT	SIZE	CATALOG #	COMPONENT
MyoCult™-SF Expansion Supplement Kit (Human)	1 Kit	05980	<ul style="list-style-type: none"><li>MyoCult™-SF Expansion 10X Supplement (Human) (2 x 10 mL)</li><li>MyoCult™-SF Attachment Substrate (100 µg)</li></ul>
MyoCult™ Differentiation Kit (Human)	200 mL	05965	<ul style="list-style-type: none"><li>MyoCult™ Differentiation Basal Medium (Human) (190 mL)</li><li>MyoCult™ Differentiation 20X Supplement (Human) (10 mL)</li><li>Animal Component-Free (ACF) Cell Attachment Substrate (1 mL)</li></ul>

## Supporting Products for Human Skeletal Muscle Research

PRODUCT	CATALOG #	APPLICATION
DMEM with 1000 mg/L D-Glucose	36253	General cell culture
EasySep™ Human CD56 Positive Selection Kit II	17855	Purification of CD56 <sup>+</sup> myoblasts from bulk human skeletal muscle cell culture
Anti-Human CD56 (NCAM) Antibody, Clone HCD56	60021	Characterization and isolation of myoblasts
Anti-Human CD45 Antibody, Clone HI30	60018	
Anti-Human CD45 Antibody, Clone 2D1	60123	

## References

1. Chal J. et al. (2016) Generation of human muscle fibers and satellite-like cells from human pluripotent stem cells in vitro. Nat Protoc 11(10): 1833–1859.
2. Xi H. et al (2017) In vivo human somitogenesis guides somite development from hPSCs. Cell Rep (18)6: 1573–1585.

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