# MyoCult™-SF Expansion Supplement Kit (Human)

**Serum-free supplement and attachment substrate for the derivation and expansion of human skeletal muscle progenitor cells (myoblasts)**

| | |
|---|---|
| Catalog #05980 | 1 Kit |
| Catalog #05982 | 10 mL |
| Catalog #05983 | 100 µg |

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## Product Description

MyoCult™-SF Expansion Supplement Kit (Human) contains a serum-free supplement and attachment substrate for the culture of primary human skeletal muscle progenitor cells (myoblasts) and human pluripotent stem cell (hPSC)-derived myoblasts. The supplement must be combined with a basal medium (DMEM with 1000 mg/L D-Glucose [Catalog #36253]; sold separately) to prepare MyoCult™-SF Expansion Medium. This medium has been optimized for the derivation and expansion of human skeletal muscle progenitor cells in vitro. For primary myoblasts, cultureware must be coated with MyoCult™-SF Attachment Substrate; for hPSC-derived myoblasts, cultureware must be coated with Corning® Matrigel® hESC-Qualified Matrix. Myoblasts cultured using MyoCult™-SF Expansion Medium can be differentiated into multinucleated myotubes using MyoCult™ Differentiation Kit (Human; Catalog #05965).

## Product Information

| PRODUCT NAME | CATALOG # | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| MyoCult™-SF Expansion Supplement Kit (Human)<br>• MyoCult™-SF Expansion 10X Supplement (Human), 2 x 10 mL<br>• MyoCult™-SF Attachment Substrate, 100 µg | 05980 | 1 Kit | Not applicable. | Not applicable. |
| MyoCult™-SF Expansion 10X Supplement (Human) | 05982 | 10 mL | Store at -20°C. | Stable for 2 years from date of manufacture (MFG) on label. |
| MyoCult™-SF Attachment Substrate | 05983 | 100 µg | Store at -20°C to -80°C. | Stable until expiry date (EXP) on label. |

## Materials Required but Not Included

| PRODUCT | CATALOG # |
|---|---|
| 10 mL syringe with 20 gauge needle | --- |
| 100 mm Dish, Non-Treated | 38045 |
| 37 µm Reversible Strainer, Large | 27250 |
| 70 µm Reversible Strainer, Large | 27260 |
| ACCUTASE™ | 07920 |
| Collagenase A, ACF | 07434 |
| D-PBS (Without Ca++ and Mg++) | 37350 |
| D-PBS with Ca++ and Mg++ | --- |
| DMEM with 1000 mg/L D-Glucose | 36253 |
| Dispase, ACF | 07446 |
| EasySep™ Red Blood Cell Lysis Buffer | 20110 |
| Falcon® Conical Tubes, 15 mL and 50 mL | 38009 and 38010 |
| Falcon® Serological Pipettes, 10 mL | 38004 |

# Preparation of Reagents and Materials

## MyoCult™-SF Expansion Medium

Use sterile technique to prepare MyoCult™-SF Expansion Medium (DMEM with 1000 mg/L D-Glucose + MyoCult™-SF Expansion 10X Supplement). The following example is for preparing 100 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw MyoCult™-SF Expansion 10X Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 10 mL of MyoCult™-SF Expansion 10X Supplement to 90 mL of DMEM with 1000 mg/L D-Glucose. Mix thoroughly.

   NOTE: If not used immediately, store MyoCult™-SF Expansion Medium at 2 - 8°C for up to 2 weeks.

   NOTE: MyoCult™-SF Expansion Medium does not contain antibiotics, but can be supplemented with penicillin-streptomycin or gentamicin at standard concentrations. When performing derivation experiments from tissue samples, supplementation with antibiotics is recommended.

## Coating Cultureware with MyoCult™-SF Attachment Substrate (For Primary Myoblasts)

Use sterile technique when coating cultureware with MyoCult™-SF Attachment Substrate.

NOTE: Use tissue culture-treated cultureware.

1. Thaw MyoCult™-SF Attachment Substrate at 2 - 8°C.

   NOTE: If not used immediately, store at 2 - 8°C for up to 3 months.
2. Add MyoCult™-SF Attachment Substrate to D-PBS with Ca++ and Mg++ at a 1 in 50 dilution.

   *For example, add 120 µL of MyoCult™-SF Attachment Substrate to 5.88 mL of D-PBS.*
3. Gently mix the diluted MyoCult™-SF Attachment Substrate. Do not vortex. Immediately use diluted MyoCult™-SF Attachment Substrate to coat cultureware. Refer to Table 1 for recommended coating volumes.

   **Table 1. Volumes Recommended for Coating Cultureware with Diluted MyoCult™-SF Attachment Substrate**

| CULTUREWARE | VOLUME OF DILUTED MYOCULT™-SF ATTACHMENT SUBSTRATE |
|---|---|
| 12-well plate | 500 µL/well |
| 6-well plate | 1 mL/well |
| T-25 $cm^2$ flask | 2.5 mL/flask |
| T-75 $cm^2$ flask | 6 mL/flask |

4. Gently rock the cultureware back and forth to spread diluted MyoCult™-SF Attachment Substrate evenly across the surface of the cultureware.
5. Seal cultureware to avoid evaporation (e.g. using Parafilm®). Incubate at 2 - 8°C overnight.

   NOTE: If not used immediately, store coated cultureware at 2 - 8°C for up to 1 week. Do not allow the culture surface to dry, as the matrix will become inactivated.
6. Immediately prior to plating cells, gently tilt the cultureware onto one side and allow the excess substrate solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
7. Plate cells immediately.

## Coating Cultureware with Corning® Matrigel® (For hPSC-Derived Myoblasts)

For complete instructions on coating cultureware with Corning® Matrigel® (Corning Catalog #354277), refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1, available at www.stemcell.com, or contact us to request a copy.

# Directions for Use

The protocols in sections B, D, and E have been validated using hPSC-derived myoblasts generated using the methods published by Chal et al. and Xi et al. When culturing hPSC-derived myoblasts, use cultureware coated with Corning® Matrigel® (see Preparation section); proceed to section B for passaging.

When culturing primary myoblasts, use cultureware coated with MyoCult™-SF Attachment Substrate; proceed to section A for derivation.

## A. DERIVATION OF MYOBLASTS FROM HUMAN SKELETAL MUSCLE TISSUE

MyoCult™-SF Expansion Supplement Kit is compatible with various methods for isolating myogenic progenitor cells from human skeletal muscle tissue, including enrichment for CD56+ cells by fluorescence-activated cell sorting (FACS) (Agley et al., Charville et al., Soriano-Arroquia et al.).

The following protocol is a basic procedure for muscle digestion and initiation of a *bulk* muscle culture (myoblasts and fibroblasts), from which myoblasts can be enriched by magnetic cell separation.

This protocol is optimized for 1 g of skeletal muscle tissue as starting material. For other amounts of tissue, further optimization may be required.

1. In a sterile 100 mm dish, finely mince human skeletal muscle tissue using tweezers and scissors. Remove and discard all fat and connective tissue attached to the muscle sample.

   NOTE: Removal of fat and connective tissue is critical for optimal muscle digestion.
2. In a 50 mL conical tube, prepare 7 mL of 630 U/mL Collagenase A, ACF diluted in DMEM with 1000 mg/L D-Glucose.
3. Transfer minced tissue to the tube prepared in step 2.
4. Secure the tube containing minced tissue horizontally on an orbital shaker in a 37°C incubator. Incubate for 1 hour with gentle rotation (approximately 30 RPM).
5. Prepare 1 mL of 4400 U/mL Collagenase A, ACF and 9 U/mL Dispase, ACF diluted in DMEM with 1000 mg/L D-Glucose.
6. After 1 hour, add 1 mL of the Collagenase/Dispase solution (prepared in step 5) to the minced tissue. Using a 10 mL serological pipette, pipette the entire volume up and down 5 - 10 times until the solution is homogenous.
7. Secure the tube containing minced tissue horizontally on an orbital shaker in a 37°C incubator. Incubate for 1 hour with gentle rotation (approximately 30 RPM).
8. Remove tube from the orbital shaker. Using a 10 mL syringe with a 20 gauge needle, draw up the sample slowly, dispense, and repeat 10 times.

   NOTE: If large undigested tissue pieces remain and cannot be pushed through the syringe easily, return the tube to the orbital shaker at 37°C for an additional 15 - 30 minutes. After this additional digestion, if material still cannot be passed through the needle, discard the undigested tissue. Skeletal muscle tissue samples from older donors may require longer digestion times.
9. Add 8 mL of MyoCult™-SF Expansion Medium to the cell suspension prepared in step 8. Vortex briefly to mix.
10. Place a 70 µm Reversible Strainer onto a new 50 mL conical tube and pass 1 mL of DMEM with 1000 mg/L D-Glucose through the strainer in order to wet the filter.
11. Pass the cell suspension through the strainer. Wash strainer with 2 mL of DMEM with 1000 mg/L D-Glucose. Retain the flow-through.
12. Place a 37 µm Reversible Strainer onto a fresh 50 mL conical tube and pass 1 mL of MyoCult™-SF Expansion Medium through the strainer in order to wet the filter.
13. Pass the flow-through from step 11 through the 37 µm strainer. Wash the strainer with 2 mL of DMEM with 1000 mg/L D-Glucose.
14. Evenly divide the flow-through into 2 x 15 mL conical tubes.
15. Centrifuge tubes at 400 x *g* for 15 minutes at 2 - 8°C. Aspirate liquid, leaving cell pellets intact.
16. Add 1 mL of EasySep™ Red Blood Cell Lysis Buffer (diluted to 1X) to each cell pellet. Incubate at room temperature (15 - 25°C) for 8 minutes.
17. Combine the contents of the 2 tubes into one 15 mL conical tube. Add 10 mL of MyoCult™-SF Expansion Medium.
18. Centrifuge the 15 mL tube at 400 x *g* for 15 minutes at 2 - 8°C. Aspirate liquid, leaving cell pellet intact.
19. Resuspend pellet in an appropriate amount of MyoCult™-SF Expansion Medium.
20. Count cells and plate at a density of 2 - 4 x 10^4 cells/cm$^2$ onto cultureware coated with MyoCult™-SF Attachment Substrate (see Preparation section).
21. Incubate at 37°C and 5% $CO_2$ until cells are 50 - 60% confluent (~4 - 7 days). Perform a full-medium change every other day.

    NOTE: It is important that cells do not become over-confluent, as this will induce premature differentiation and cell cycle exit.
22. Proceed to section B for passaging.

### B. PASSAGING MYOBLASTS

Passage myoblasts when cells are 50 - 60% confluent; it is important that cells do not become over-confluent.

The following protocol is for passaging human myoblasts in one well of a 6-well tissue culture plate (e.g. Catalog #38015). If using alternative cultureware, adjust volumes accordingly.

1. Wash cells with 2 mL of D-PBS (Without Ca++ and Mg++). Remove D-PBS.
2. Add 1 mL of ACCUTASE™ and incubate at 37°C for 6 - 8 minutes. Tap the flask to detach cells.
3. Add 2 mL of MyoCult™-SF Expansion Medium and transfer cells to a 15 mL conical tube.
4. Centrifuge at 300 x *g* for 10 minutes. Remove and discard the supernatant.
5. Resuspend the cell pellet in MyoCult™-SF Expansion Medium.
6. Count cells and plate at a density of 5 x 10^3 cells/cm$^2$ onto cultureware coated with either MyoCult™-SF Attachment Substrate (primary myoblasts) or Corning® Matrigel® (hPSC-derived myoblasts).

### C. PURIFYING MYOBLASTS

After 1 - 2 passages, primary myoblasts can be purified either by FACS with selection for CD56+ cells or by magnetic cell separation with EasySep™ Human CD56 Positive Selection Kit II (Catalog #17855) using the protocol described in Document #10000000694 (available at www.stemcell.com, or contact us to request a copy). Purified myoblasts can be subsequently expanded and passaged.

NOTE: Purifying primary myoblasts is recommended prior to differentiation (section E).

### D. CRYOPRESERVING MYOBLASTS

Myoblasts can be cryopreserved in serum-free conditions using CryoStor® CS10 (Catalog #07930). In order to retain differentiation potential, cryopreserve myoblasts no later than passage 5.

### E. DIFFERENTIATING MYOBLASTS

Purified primary myoblasts and hPSC-derived myoblasts can be differentiated into multinucleated myotubes using MyoCult™ Differentiation Kit (Catalog #05965). For optimal myotube formation, induce differentiation at earlier passages (< passage 6). Differentiation potential could be reduced or lost at higher passages.

## Related Products

For related products, including antibodies, small molecules, and cultureware, visit www.stemcell.com/myogenicworkflow, or contact us at techsupport@stemcell.com.

## References

Agley CC et al. (2015) Isolation and quantitative immunocytochemical characterization of primary myogenic cells and fibroblasts from human skeletal muscle. J Vis Exp (95): 52049.

Chal J et al. (2016) Generation of human muscle fibers and satellite-like cells from human pluripotent stem cells in vitro. Nature Protocols (11): 1833–50.

Charville GW et al. (2015) Ex vivo expansion and in vivo self-renewal of human muscle stem cells. Stem Cell Reports 5(4): 621–32.

Soriano-Arroquia A et al. (2017) Preparation and culture of myogenic precursor cells/primary myoblasts from skeletal muscle of adult and aged humans. J Vis Exp (120): e55047.

Xi H et al. (2017) In vivo human somitogenesis guides somite development from hPSCs. Cell Reports 18(6): 1573–85.