MyoCult™ Expansion 10X Supplement (Mouse)

Serum-containing supplement for the expansion of mouse skeletal muscle progenitor cells (satellite cells)

Catalog #05985 10 mL

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Product Description

MyoCult™ Expansion 10X Supplement (Mouse), when combined with DMEM/F-12 to prepare MyoCult™ Expansion Medium, is for the culture of mouse skeletal muscle progenitor cells (satellite cells). MyoCult™ Expansion Medium has been optimized for the expansion of mouse skeletal muscle satellite cells isolated by fluorescence-activated cell sorting (FACS), as well as for culturing single isolated myofibers grown in suspension. Satellite cells cultured using MyoCult™ Expansion Medium can be differentiated into multinucleated myotubes.

For preparation of MyoCult™ Expansion Medium, DMEM/F-12 with 15 mM HEPES (Catalog #36254) is required in addition to MyoCult™ Expansion 10X Supplement. For expansion of satellite cells, cultureware must be coated with a matrix such as Corning Matrigel® Basement Membrane Matrix (Corning Catalog #356234).

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
MyoCult™ Expansion 10X Supplement (Mouse)	05985	10 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.

Preparation of Reagents and Materials

MyoCult™ Expansion Medium

Use sterile techniques to prepare MyoCult™ Expansion Medium (DMEM/F-12 with 15 mM HEPES + MyoCult™ Expansion 10X Supplement). The following example is for preparing 100 mL of medium. If preparing other volumes, adjust accordingly.

1. Thaw MyoCult™ Expansion 10X Supplement (Mouse) at room temperature (15 - 25°C) or at 2 - 8°C overnight. 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 Supplement to 90 mL of DMEM/F-12. Mix thoroughly.

   NOTE: If desired, add penicillin-streptomycin or gentamicin at standard concentrations.

   NOTE: If not used immediately, store MyoCult™ Expansion Medium at 2 - 8°C for up to 4 weeks.

Coating Cultureware

For expansion of mouse skeletal muscle satellite cells, coat tissue culture-treated cultureware with Corning® Matrigel® (Corning Catalog #356234). Alternatively, coat cultureware with either Collagen Solution (Catalog #04902) or MyoCult™-SF Attachment Substrate (Catalog #05983).
Use sterile techniques when coating cultureware.

NOTE: If culturing single isolated myofibers grown in suspension, cultureware does not require coating.

1. Thaw Matrigel® on ice.
2. Dilute Matrigel® 1 in 100 with cold (2 - 8°C) DMEM/F-12 and mix gently.
3. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. Refer to Table 1 for recommended volumes.

Table 1. Recommended Volumes of Diluted Matrigel® Solution for Coating Various Cultureware

TISSUE CULTURE-TREATED CULTUREWARE	VOLUME OF DILUTED MATRIGEL® SOLUTION
96-well plate	100 µL/well
48-well plate	200 µL/well
24-well plate	350 µL/well
12-well plate	500 µL/well
6-well plate	1 mL/well
100 mm dish	6 mL/dish
T-75 cm^2 flask	8 mL/flask

4. Gently rock the cultureware back and forth to spread the solution evenly across the surface of the cultureware.
5. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the Matrigel® solution (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature (15 - 25°C) for 30 minutes before proceeding to the next step.
6. Gently tilt the cultureware onto one side and allow the excess solution to collect at the edge. Carefully remove excess solution using a serological pipette or by aspiration, ensuring that the coated surface is not scratched.
7. Plate cells immediately (see Directions for Use).

Directions for Use

The following protocols are for expansion of satellite cells isolated from mouse skeletal muscle tissue (section A) or for culture of single isolated myofibers grown in suspension (section B).

A. EXPANSION OF SATELLITE CELLS ISOLATED FROM MOUSE SKELETAL MUSCLE TISSUE

1. Isolate satellite cells from mouse skeletal muscle tissue by methods such as direct FACS isolation.[1,2,3]
2. Resuspend satellite cells with MyoCult™ Expansion Medium and seed cells at a density of 1,500 - 2,000 cells/cm^2 into matrix-coated cultureware (see Preparation section).
3. Incubate cells at 37°C and 5% CO_2 for 3 days. Perform a full medium change. Continue incubation, performing a full medium change every other day.
4. Passage cells once they reach 60 - 70% confluency (approximately 4 - 6 days), as follows:
   a. Wash cells with D-PBS (Without Ca++ and Mg++). Remove D-PBS.
   b. Add Trypsin-EDTA (0.25%; Catalog #07901) and incubate at 37°C for 2 - 4 minutes. Tap vessel to detach cells.
   c. Add MyoCult™ Expansion Medium to resuspend detached cells; this will stop the enzymatic activity.
   d. Seed cells at a > 1:5 split ratio (> 5000 cells/cm^2) into matrix-coated cultureware.

      NOTE: Satellite cells typically remain proliferative for 1 - 2 passages.

      NOTE: Cells can be seeded at a higher density (> 10,000 cells/cm^2) for downstream differentiation assays.

B. CULTURE OF SINGLE ISOLATED MYOFIBERS GROWN IN SUSPENSION

NOTE: Cultureware does not require coating when using myofiber suspension cultures.

1. Add single isolated myofibers into MyoCult™ Expansion Medium in appropriately sized tissue culture-treated cultureware.
2. Incubate at 37°C and 5% CO_2 for up to 96 hours.

   NOTE: For methods for isolating single myofibers from suspension cultures, refer to Pasut et al. For assaying satellite cells from single isolated myofibers grown in suspension, including immunofluorescent staining, refer to Brun et al.

References

1. Liu L et al. (2015) Isolation of skeletal muscle stem cells by fluorescence-activated cell sorting. Nat Protoc 10(10): 1612–24.
2. Gromova A et al. (2015) FACS-based satellite cell isolation from mouse hind limb muscles. Bio-protocol 5(16): e1558.
3. Motohashi N et al. (2014) Isolation, culture, and transplantation of muscle satellite cells. J Vis Exp (86): 50846.
4. Pasut A et al. (2013) Isolation and culture of individual myofibers and their satellite cells from adult skeletal muscle. J Vis Exp (73): e50074.
5. Brun CE et al. (2018) Single EDL myofiber isolation for analyses of quiescent and activated muscle stem cells. Methods Mol Biol 1686: 149–59.