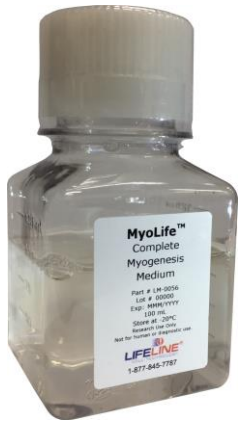
MyoLife™ Complete Myogenesis Differentiation Medium Instruction Sheet



Safety and Use Statement

This product is for Research Use Only. This product is not approved for human or veterinary use or for use in *in vitro* diagnostics or clinical procedures.

Lifeline recommends storing cryopreserved vials in liquid nitrogen vapor phase. Handle cryopreserved vials with caution. Always wear eye protection and gloves when working with cell cultures. Aseptically vent any nitrogen from cryopreserved vials by carefully loosening the vial cap in a biosafety cabinet prior to thawing the vials in a water bath. If vials must be stored in liquid phase, the vials should be transferred to vapor phase storage or -80°C for up to 24 hours prior to being thawed.

MyoLife Myogenesis Differentiation Medium is optimized for the differentiation of Human Skeletal Muscle Satellite Cells into fused multinucleated myotubules.

Medium Storage

MyoLife Medium should be stored at -20°C until ready to use. For long-term storage, it should be stored at -70°C or lower. Once thawed, it should be stored at 2 to 8°C for up to three weeks. Do not use product beyond expiration date. Multiple freeze/thaw cycles are not recommended. Users should take care to protect medium from extended exposure to light.

Product	Part No.	Volume	Final Concentration in Supplemented Medium	Storage
MyoLife Complete Myogenesis Differentiation Medium	LM-0056	100 mL	2% (v/v) Horse serum 10 mM L-Glutamine	-20°C or 2-8°C once thawed

Other Recommended Products	Part No.	Unit	Components	Storage
Human Skeletal Muscle Satellite Cells	FC-0091	500,000 cells		-150°C
StemLife™ Sk Medium Complete Kit	LL-0069	Kit	StemLife Basal Medium StemLife Sk LifeFactors® Kit	2-8°C when prepared
Antimicrobial Supplement: Gentamicin and Amphotericin B *(Provided with purchase of LL-0069)*	LS-1104	0.5 mL	Gentamicin 30 µg/mL Amphotericin B 15 ng/mL	-20°C
TrypKit™	LL-0013	Kit	PBS 0.05% Trypsin/0.02% EDTA Trypsin Neutralizing Solution	RT -20°C -20°C
Optional Supplements	**Part No.**	**Volume**	**Concentrations of Supplement**	**Storage**
Phenol Red Supplement	LS-1009	1 mL	33 mM	RT

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Basic Aseptic Technique

Medium, LifeFactors®, and cells should only be used in an aseptic environment, a Class II biological safety cabinet with front access and filtered laminar airflow, or an equivalent device. Always wear gloves and eye protection when working with these materials. Wipe or spray all bottles and vials with 70% ethanol or isopropanol, especially around the area of the cap, before placing them in the biological safety cabinet. Allow these surfaces to dry completely before opening the bottle or vials. Transfer cells, medium, or LifeFactors with disposable sterile pipettes. Do not mouth pipette! Take up the volume needed into the pipette, being careful not to touch the sterile tip to the rim of the container or any other surface. Close the container and open the container into which the transfer is being made, again being careful not to touch any surfaces with the sterile tip. Transfer the material and close the container. Wash your hands before and after working with cell cultures. Do not block airflow in a laminar flow hood as this may compromise sterility. Ensure that biological safety cabinets are certified routinely and that the HEPA filters are replaced regularly.

Pre-warming Medium

If using less than 100 mL of medium, Lifeline® recommends warming only the volume needed in a sterile conical tube. Repeated warming of the entire bottle over extended periods will cause degradation and reduced shelf life of the medium. When warming the entire bottle of medium, Lifeline recommends using a Lifeline water bath sleeve (included with medium) to help protect the medium from contaminants in the 37°C water bath. Medium will warm to 37°C in 10 to 30 minutes, depending on the volume. Do not leave medium in water bath for extended periods.

Thawing and Plating Cryopreserved Cells

Pre-warm fully supplemented StemLife™ Sk Medium. See StemLife Sk instruction sheet for details on how to prepare the medium.

Remove vial from dewar and check the cap to be sure that the vial is securely sealed. Spray the vial with 70% ethanol or isopropanol and transfer it to a biosafety cabinet. Allow it to dry thoroughly and carefully loosen the cap to vent any liquid nitrogen that may have entered the vial. Recap the vial and hold only the bottom half of the vial in a 37°C water bath for approximately one minute or until vial is almost completely thawed—a small amount of ice should still be visible. To avoid potential contamination, do not allow the vial cap to make contact with the water. Do not over thaw as this may damage the cells. Dry the vial, spray the exterior of the vial with 70% ethanol or isopropanol and place the vial in a biological safety cabinet and allow it to dry. Carefully remove the cap to avoid contamination or splatter. Gently resuspend the cells in the vial using a 1 or 2 mL sterile pipette. Do not centrifuge; the cells may be directly plated from the vial. Plate cells into pre-warmed fully supplemented medium (respective to cell type) in the desired culture vessel at a density of 5,000 cells per cm^2. Flasks with vented caps, commonly referred to as filter caps, are strongly recommended. Gently rock the culture vessel from side to side and front to back to evenly distribute cells within the vessel. Place seeded culture vessel in a 37°C, 5% CO_2 incubator. Re-feed the cells after they have attached (approximately 4 to 36 hours after inoculation) to remove cryopreservation reagents.

Recommended Feeding Guidelines for Undifferentiated Expansion of HSkMC

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of fresh supplemented medium. ***NOTE: Regular cell feeding is critical, as cells may spontaneously differentiate if nutrients become depleted.***
Most cultures which are 50% confluent will be ready for passage within two days and should be fed with 7 to 8 mL of supplemented medium.
Do not use more than 10 mL of medium per 25 cm^2 of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

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Passaging Undifferentiated HSkMC

HSkMC may be passaged when the culture is 70 to 90% confluent and actively proliferating. Lifeline® recommends passaging HSkMC before reaching confluence since post-confluent cells exhibit morphological changes, slower growth and differentiation.

Lifeline recommends using the TrypKit™ Subculture Reagent Kit (LL-0013). Aspirate the medium from the culture vessel. Rinse the flasks with Lifeline's Phosphate Buffered Saline (PBS; CM-0001) by adding at least 1 mL of PBS per each 5 cm^2 and gently tilting the flask to cover the surface with PBS. Aspirate the PBS from the culture vessel, repeat the rinse if desired. Add at least 2 mL of Lifeline's 0.05% Trypsin/0.02% EDTA (CM-0017) per each 25 cm^2 to the vessel. Swirl gently to ensure all cells are coated with the Trypsin/EDTA. Observe the cells carefully under the microscope. When the cells round up they are ready to be released. This normally takes from 2 to 3 minutes depending on the confluence of the cells. Do not over trypsinize as this may damage the cells. Detach the cells by gently striking the culture vessel against your hand several times. Observe the cells under the microscope to be sure they have become detached. Once the cells are fully detached, add Lifeline's Trypsin Neutralizing Solution (TNS; CM-0018) using a volume equal to the amount of Trypsin/EDTA that was originally used. Gently swirl to ensure all of the trypsin solution is neutralized. Using aseptic laboratory techniques, pipette the cells into a sterile centrifuge tube. Collect the remaining cells by rinsing the culture vessel with at least 1 mL of PBS per each 5 cm^2 and pipetting the cells into the sterile centrifuge tube. Check culture vessel under the microscope for cells still attached and repeat steps if necessary to retrieve all the cells from the vessel. All steps must be completed under aseptic conditions in a biological safety cabinet.

Centrifuge the cells at 150 x *g** for 3 to 5 minutes. For best results, calculate speed for individual centrifuge type. Time may also be centrifuge dependent. Do not over centrifuge cells as this will cause cell damage. After centrifugation, the cells should form a clean loose pellet. Please consult Lifeline's technical service department if issues arise from trypsinization or centrifugation.

Aspirate the neutralized trypsin solution from the centrifuge tube and resuspend the cell pellet in pre-warmed culture medium (respective to cell type) by gently pipetting up and down with a 2 or 5 mL pipette. Count cells using a hemacytometer. If expansion of undifferentiated cells is desired, re-plate at 5,000 cells per cm^2 **(or use a split ratio of 1:6 to 1:10)** in vessel containing the respective pre-warmed culture medium. If differentiation of cells is desired, see instructions on the following page.

***To calculate RCF ('x *g*')** **RCF = 0.00001118 x $(rpm)^2$ x r**
r = rotational radius in centimeters
rpm = rotations or revolutions per minute

To Initiate Myogenesis Differentiation

Standard Calculation for Plating of Cells for Myogenesis

Gently re-suspend the cells evenly in the respective pre-warmed complete StemLife™ Sk Culture Medium. Using a clean hemacytometer and aseptic technique, remove 25 µL of the cell suspension to a separate tube, such as a microcentrifuge tube. Add 75 µL of 0.4% Trypan Blue solution to the cell suspension in the microcentrifuge tube and allow it to sit for up to 1 to 5 minutes. Place 10 µL of the cell suspension into each chamber of the hemacytometer. Count a minimum of 4 quadrants on the hemacytometer (see diagram below). Dead and dying cells are permeable to Trypan Blue, viable cells will not be blue. For accurate cell counts, optimal number of cells per quadrant should be
25 to 75 cells. After counting the cells, calculate the average of the number of quadrants counted. Take the cell count average and multiply by the dilution factor and by 10^4 to get the number of cells per mL. Multiply the desired inoculation density (40,000 to 50,000 viable cells per cm^2) by the surface area of the well(s) to be inoculated. This will give you the total number of cells to inoculate one well. Divide the number of cells needed to inoculate the well(s) by the total number of cells in the cell suspension. This will give you the volume of cell suspension with which to inoculate the well(s). Inoculate the cells into the well(s) of the culture vessel(s) prepared with pre-warmed culture medium. Mix gently to evenly distribute the cells and place culture vessel(s) into the incubator at

37°C, 5% CO_2. (See TrypKit Instructions for more detailed cell counting instructions.)

Sample calculation:

Average viable cells per quadrant = 31

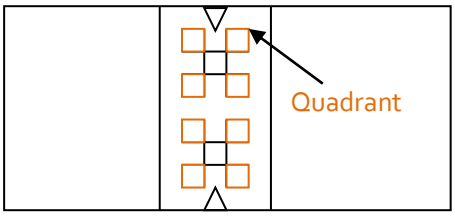
31 cells/quadrant x 10,000 quadrants/mL x 4 (dilution factor) = 1,240,000 cells/mL

Inoculating a 6-well plate at 18,000 cells/cm² (if using a Corning® brand 6-well plate):

18,000 cells/cm² x 9.5 cm²/well = 171,000 cells/well

Calculate volume of cell suspension required to inoculate each well with:

171,000 cells/well divided by 1,240,000 cells/mL = 0.138 mL/well



Fibronectin Coating of Culture Surface Prior to Seeding for Differentiation

Due to the increased cell tension that occurs during the differentiation process, Lifeline® recommends pre-coating the culture surface with a fibronectin coating solution. This is an optional step, but is highly recommended.

Seeding Density for Initiating Myogenesis

Cell Part Number	Cell Type	Seeding Density (cells/cm²)	Expansion Medium	Expansion Medium Part Number	Differentiation Medium	Differentiation Medium Part Number
FC-0091	HSkMC	40,000 to 50,000	StemLife Sk Medium	LL-0069	MyoLife™ Medium	LM-0056

Format for Cell Density and MyoLife Medium Volume for Standard Well Plates:

Plate Format	Seeding Density (cells/well)	Medium Volume	Feeding Schedule
6-well	380,000 to 475,000	2 mL	Every two days, for up to one week
12-well	152,000 to 190,000	1 mL	Every two days, for up to one week
24-well	76,000 to 95,000	0.5 mL	Every two days, for up to one week
48-well	38,000 to 47,000	0.3 mL	Every two days, for up to one week
96-well	13,200 to 16,500	100 µL	Every two days, for up to one week

MyoLife™ Complete Myogenesis Differentiation Medium Preparation

MyoLife Complete Myogenesis Differentiation Medium contains all the factors necessary to support the differentiation of HSkMC into fused multinucleated myotubules. MyoLife Medium is prepared by thawing and warming to 37°C.

MyoLife Complete Myogenesis Medium contains no antimicrobials and no phenol red. Antimicrobials are not required, but are recommended during differentiation due to the duration of culture and the frequency of handling of the cultures. Antimicrobials and phenol red may be purchased separately from Lifeline®.

Plating and Culturing Cells for Myogenesis

Using the passaging and cell counting instructions on pages 3 and 4, inoculate HSkMC in the StemLife™ Sk Culture Medium. (See chart on page 4 for cell density and medium volumes, respective to multi-well plate format). Gently rock the multi-well plate(s) from side to side and front to back to evenly distribute cells within the well(s). Never swirl the plate(s) as this will result in uneven distribution of cells. Place inoculated plate(s) in a 37°C, 5% CO_2 incubator.

24 hours after seeding the multi-well plate(s) with HSkMC, initiate myogenesis by aspirating culture medium from each well. Rinse the well(s) once with Lifeline's PBS (CM-0001) by adding a full volume of PBS per well (see chart above for volume per well). Aspirate the PBS from the well(s). Add a full volume of MyoLife Complete Myogenesis Differentiation Medium to each well containing cells (see chart on page 4 for volume per well). Return the plate(s) to a 37°C, 5% CO_2 incubator.

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The monolayer of cells is fragile, under tension, and must be handled with extreme care or it may detach from the plate(s): Do not tilt plate(s) during aspiration. Once differentiation has been initiated, never completely aspirate a well and expose the monolayer to air. When adding MyoLife Complete Myogenesis Differentiation Medium, pipette the medium gently down the side of the well.

The culture(s) should be re-fed every 2 days using pre-warmed MyoLife Complete Myogenesis Differentiation Medium. Re-feeding is performed by aspirating half of the medium volume from each well and adding a full volume of pre-warmed MyoLife Medium to each well (see chart on page 4 for respective myogenesis medium volumes).

After 4 to 7 days of differentiation, myogenesis is complete and cells should be fixed and stained.

Quick Steps for Differentiation of HSkMC to Fused Multinucleated Myotubules

Pre-Differentiation

1. **Always wash hands before and after working with cell cultures.**
2. **Always wear eye protection and gloves when working with cell cultures.**
3. **When working with cells or medium, always use a certified biological safety cabinet.**
4. **Store cryopreserved cells in liquid nitrogen, vapor phase.**
5. **Handle cryopreserved vials with caution. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing in a water bath.**
6. Feed cells using pre-warmed Lifeline® culture medium according to feeding guidelines.
7. Expand HSkMC in the StemLife™ Sk Culture Medium as detailed on page 3.

Differentiation

Day 0

1. When cells are 70 to 90% confluent and actively proliferating, passage cells using Lifeline subculture reagents (as detailed on page 3).
2. Pre-coat multi-well plate(s) with a fibronectin coating solution. (Optional, but highly recommended).
3. Resuspend pellet in the complete StemLife Sk Culture Medium.
4. Inoculate cells into multi-well plate(s) at 40,000 to 50,000 cells/cm^2 (see the chart on page 4 for details).
5. Incubate multi-well plate(s) at 37°C, 5% CO_2.

Day 1

6. Gently aspirate the culture medium from each well and rinse with PBS.
7. Replace the PBS with MyoLife™ Complete Myogenesis Differentiation Medium, respective to the multi-well plate size (see the chart on page 4).
8. Incubate multi-well plate(s) at 37°C, 5% CO_2.

Day 2 Through Day 7

9. Gently aspirate half the volume of medium in each well.
10. Gently add a full volume of MyoLife Complete Myogenesis Medium per well every 2 days, respective to the multi-well plate size (see the chart on page 4).
11. Incubate multi-well plate(s) at 37°C, 5% CO_2.

Day 7

12. Differentiation is complete.
13. Fix the cells and stain.

For any question on cell handling, differentiation, or staining; please contact technical service at 877.845.7787.

We are here to help.

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