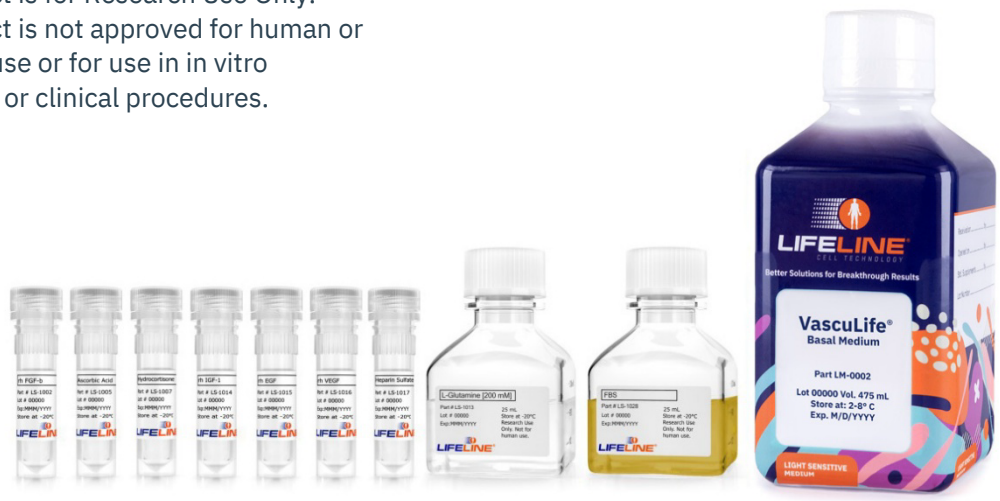
Instruction Sheet

VascuLife® VEGF-Mv Microvascular Endothelial Cell Culture Medium

LL-0005
LM-0002
LS-1029

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.



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Safety and Use Statement

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Medium Storage

VascuLife Basal Medium should be stored at 2 to 8°C. Lifeline's packaging helps protect VascuLife Basal Medium from light damage; however, users should take care to protect basal medium from extended exposure to light.

VascuLife VEGF-Mv LifeFactors™ should be stored at -20°C or lower. For long-term storage, LifeFactors should be stored at -70°C or lower. Thaw frozen LifeFactors immediately prior to adding to the basal medium. L-Glutamine (LS-1013) should be warmed to 37°C in a water bath and mixed to dissolve any precipitates before use. Do not use products beyond expiration date.

Medium Preparation

VascuLife Basal Medium contains no growth factors, antimicrobials, or phenol red. To support cell proliferation, VascuLife VEGF-Mv LifeFactors (LS-1029) must be added to the basal medium as instructed on the following page. Lifeline offers antimicrobials and phenol red; however, they are not required for cell proliferation. A vial of Gentamicin and Amphotericin B (GA; LS-1104) is provided with the purchase of VascuLife VEGF-Mv Medium Complete Kit (LL-0005) for your convenience. Phenol Red (LS-1009) may be purchased, but is not required.

Product	Part No.	Volume	Final Concentrations in Supplemented Medium	Storage
VascuLife VEGF-Mv Medium Complete Kit, (VascuLife Basal Medium, VascuLife VEGF-Mv LifeFactors Kit)	LL-0005			2-8°C when prepared
VascuLife Basal Medium	LM-0002	475 mL		2-8°C
VascuLife VEGF-Mv LifeFactors Kit	LS-1029			-20°C
rh FGF basic LifeFactor	LS-1002	0.5 mL	5 ng/mL	-20°C
Ascorbic Acid LifeFactor	LS-1005	0.5 mL	50 µg/mL	-20°C
Hydrocortisone Hemisuccinate LifeFactor	LS-1007	0.5 mL	1 µg/mL	-20°C
L-Glutamine LifeFactor	LS-1013	25 mL	10 mM	-20°C
rh IGF-1 LifeFactor	LS-1014	0.5 mL	15 ng/mL	-20°C
rh EGF LifeFactor	LS-1015	0.5 mL	5 ng/mL	-20°C
rh VEGF LifeFactor	LS-1016	0.5 mL	5 ng/mL	-20°C
Heparin Sulfate LifeFactor	LS-1017	0.5 mL	0.75 U/mL	-20°C
FBS LifeFactor	LS-1028	25 mL	5%	-20°C
Antimicrobial Supplement: Gentamicin and Amphotericin B *(Provided with purchase of LL-0005)*	LS-1104	0.5 mL	Gentamicin 30 µg/mL Amphotericin B 15 ng/mL	-20°C

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Optional Supplements	Part No.	Volume	Concentrations of Supplement	Storage
Phenol Red Supplement	LS-1009	1 mL	33 mM	RT

Cells Supported by VascuLife® VEGF-Mv Cell Culture Medium:

- Human Coronary Artery Endothelial Cells (FC-0032)
- Human Dermal Microvascular Endothelial Cells, Adult (FC-0039)
- Human Dermal Microvascular Endothelial Cells, Neonatal (FC-0042)
- Human Lung Microvascular Endothelial Cells (FC-0058)

Basic Aseptic Technique

Medium and LifeFactors™ 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 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.

Adding LifeFactors

When supplementing basal medium, Lifeline® recommends the use of a suitable pipette to remove the exact volume of growth factor or supplement from each LifeFactor (as stated in the Lifeline product instruction sheet). As part of Lifeline's commitment to the success of your research, LifeFactors are manufactured with a slight overfill to ensure that the full labeled volume is present. Lifeline's cells will still perform within guaranteed culture specifications if the entire volume of growth factor or supplement is directly added to the basal medium. However, for consistent performance results, please add the exact volume of growth factor or supplements as per the table provided in these instructions.

All procedures should be executed using aseptic technique (see section on basic aseptic technique). VascuLife VEGF-Mv LifeFactors (LS-1029) are sufficient to supplement one 475 mL bottle of VascuLife Basal Medium. LifeFactors should be thawed and mixed immediately prior to supplementation. L-Glutamine (LS-1013) should be warmed to 37°C in a water bath and mixed to dissolve any precipitates before use. Mix supplemented medium by gently pipetting up and down with a large volume pipette (25 or 50 mL) or gently invert the tightly closed 500 mL bottle. Do not shake or froth the medium. The supplemented medium may be stored at 2 to 8°C for up to two weeks.

Pre-warming Medium

If using less than 100 mL of complete 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. 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 of time.

Recommended Feeding Guidelines:

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.
Most cultures that are 50% confluent will be ready for passage within 2 days and should be fed with 7 to 8 mL of supplemented medium.
Do not use more than 10 mL of supplemented medium per 25 cm^2 of culture surface to ensure that the media 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).

Optional Supplements

Phenol Red:

Phenol red is a pH indicator that is not required in cell culture and may adversely influence the behavior of some cell types. Medium with phenol red will appear more yellow than red in acidic conditions and will appear more purple than red in basic conditions.

If you wish to add phenol red to Lifeline® medium, a 1 mL LifeFactor of water-soluble phenol red (LS-1009) may be purchased separately. Addition of 0.5 mL of the phenol red to 500 mL of medium will yield a 33 µM solution of phenol red and will change the medium to a strong red to reddish-purple color. The phenol red supplement may be stored at room temperature.

Gentamicin and Amphotericin B (GA):

A vial of GA (LS-1104) is provided with the purchase of VascuLife® VEGF-Mv Medium Complete Kit (LL-0005) for your convenience. The use of GA is recommended to inhibit potential fungal or bacterial contamination of eukaryotic cell cultures. GA is best stored at -20°C; it should only be thawed once and stored at 4°C for a maximum of two weeks after thaw. Addition of 0.5 mL of GA to 500 mL of media will provide an effective concentration to inhibit bacterial and fungal cell division.

The Lifeline Guarantee

Lifeline's rigorous quality control ensures sterility and performance to standardized testing criteria. Upon request, we will provide lot-specific QC test results, material safety data sheets, and certificates of analysis. See complete guarantee/warranty statement at lifelinecelltech.com or contact your technical representative for more information.

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For any question on medium supplementation or cell feeding guidelines; please contact technical service at 877.845.7787. We are here to help.

Notes:

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