



# TrailBio<sup>®</sup> Endothelial Cells User Instructions

## Product Description

This protocol provides instructions to thaw, plate, and expand functional human endothelial cells under optimized culture conditions that support cell attachment, survival, and proliferation.

**Table 1: Components**

Component Name	Size	Quantity	Storage	Catalog Number
TrailBio <sup>®</sup> Endothelial cells	1 ml	1 vial (1M Cells)	LN2, Shipped on LN2	ME010101021

### Notes

- Store components individually at the recommended storage conditions. Use cells within 6 months from date of purchase. Use medium and supplements according to the manufacturer's instructions.
- TrailBio<sup>®</sup> Endothelial Cells can be expanded for a maximum of 3 passages while maintaining expected endothelial marker expression. Passaging is recommended just before or at the moment of confluence. Do not allow cells to become overconfluent, as this may lead to loss of endothelial phenotype.

## 1. Before You Begin: Critical Success Factors

Follow the instructions below to ensure optimal performance and results.

### Initial Checklist:

1. Confirm that cell vials are undamaged and frozen upon receipt.
2. Immediately transfer cell vials to liquid nitrogen (LN2) vapor phase upon receipt. Do not store at -70 °C as this can reduce viability.
3. Read this entire protocol before starting any experimental work.
4. Use only the recommended reagents and materials listed below.
5. Use proper sterile cell culture techniques throughout.

## 2. Materials & Equipment Required (Not Included)

**Table 2: Materials required but not included**

Name	Recommended Vendor	Catalog Number
Fibronectin	Corning	354008
Sterile 15 ml conical tubes	Various	Various
DPBS without Ca <sup>2+</sup> and Mg <sup>2+</sup>	ThermoFisher Scientific	14190094
Tissue culture vessels	Various	Various
Vasculife® VEGF Endothelial Medium Complete Kit	LifeLine Cell Technology	LL-0003
VEGF-165	ThermoFisher Scientific (Peprotech)	100-20
SB431542	MedChemExpress	HY-10431
StemPro™ Accutase™ Cell Dissociation Reagent	ThermoFisher Scientific	A1110501

## 3. Culture Plate Coating (Day -1 or 0)

1. Reconstitute fibronectin according to the manufacturer's instructions and prepare a stock solution.
2. Dilute the fibronectin stock in sterile DPBS (without Ca<sup>2+</sup> and Mg<sup>2+</sup>) to the working concentration specified in Table 3 for the selected vessel format.
3. Apply the corresponding coating volume to achieve a final coating density of 1 µg/cm<sup>2</sup>.

**Table 3: Coating Volumes and Vessels**

Plate Format	Surface Area / Well	Coating Volume
T-25 flask	25 cm <sup>2</sup>	2.5 ml / flask
6 well plate	9.6 cm <sup>2</sup>	1.2 ml / well
12 well plate	3.9 cm <sup>2</sup>	440 µl / well
24 well plate	1.9 cm <sup>2</sup>	240 µl / well
96 well plate	0.32 cm <sup>2</sup>	50 µl / well

4. Ensuring the entire surface is coated, incubate culture vessel for at least 1 hour at room temperature.
5. If not used immediately, fibronectin-coated vessels may be wrapped in parafilm and stored at 4 °C for up to 1 week.

**Note:** Coated vessels stored at 4 °C should be incubated for 1 hour at room temperature prior to use. Do not use coated vessels if coating solution shows signs of evaporation.

## 4. VascuLife® VEGF Endothelial Cell Culture Medium Preparation (Day 0)

1. Prepare VascuLife® VEGF Endothelial Cell Culture Medium according to the manufacturer's instructions.  
**Note:** Prepared VascuLife® VEGF Endothelial Cell Culture Medium can be stored at 2 °C to 8 °C for up to two weeks, protected from light. Refer to the manufacturer's instructions for more information.
2. Add the components listed in Table 4 to the prepared VascuLife® medium to make 'Maintenance Medium' required for up to 7 days of culture depending on your application.  
**Note:** Maintenance Medium can be stored at 2 °C to 8 °C for up to 7 days, protected from light.

**Table 4: Maintenance Medium Preparation**

Component	Concentration
VEGF-165*	20 ng/ml*
SB431542	10 µM

\*VascuLife® VEGF Endothelial Cell Culture Medium contains VEGF; adding 20 ng/ml VEGF-165 brings the final concentration to 25 ng/ml.

## 5. Thawing and Plating Cells (Day 0)

1. Bring the required volume of Maintenance Medium to room temperature. Do not warm to 37 °C.
2. Prepare fibronectin coated plates per section 3  
**Note:** Pre-made coated vessels stored at 4 °C should be incubated for 1 hour at room temperature prior to use.
3. Add 2 ml of room temperature Maintenance Medium to a 15 mL conical tube.
4. Remove TrailBio® Endothelial Cells from liquid nitrogen and swirl the vial in a 37 °C water bath until a small ice pellet remains.  
**Note:** Do not submerge the vial.
5. Coat a pipette tip with Maintenance Medium, then transfer the cells dropwise into the prepared 15 ml conical tube.
6. Gently wash the cryovial with 1 ml of Maintenance Medium and transfer to the 15 ml conical tube containing the cells.
7. Add an additional 2 ml of Maintenance Medium to the 15 ml conical containing the cells for a total volume of 6 ml.
8. Centrifuge the cell mixture for 5 minutes at 300 x g.
9. Aspirate the supernatant leaving ~100 µl to avoid disturbing the cell pellet.
10. Resuspend the cell pellet in 4 ml of Maintenance Medium.
11. Remove a sample of the cell suspension and perform a cell count using a hemocytometer with trypan blue staining. Each vial should contain  $\geq 1$  million viable cells.  
**Note:** Vials contain excess cells to ensure sufficient viable cells for this protocol. Please perform a cell count before plating.
12. Add Maintenance Medium to the cells to achieve a target density of 10,000 cells/cm<sup>2</sup>.
13. Refer to Table 6 for seeding volumes and cell counts.
14. Aspirate fibronectin from the pre-coated culture vessel and immediately plate the cells.
15. Gently move the vessel in a T-motion to ensure even distribution.
16. Incubate at 37 °C, 5% CO<sub>2</sub>.

**Table 6: Plating & Feeding Volume Reference**

Plate Format	Surface Area / Well	Total Cells	Plating/Feeding Volume
T-25 flask	25 cm <sup>2</sup>	250,000 / flask	5 ml
6 well plate	9.6 cm <sup>2</sup>	96,000 / well	2 ml
12 well plate	3.9 cm <sup>2</sup>	39,000 / well	1 ml
24 well plate	1.9 cm <sup>2</sup>	19,000 / well	500 µl
96 well plate	0.32 cm <sup>2</sup>	3,200 / well	100 µl

## 6. Cell Maintenance (Day 2)

1. Bring required volume of Maintenance Medium to room temperature. Do not warm to 37 °C.
2. Perform a full medium change 48 hours post-thaw and every 48 hours thereafter, using the volumes listed in table 6.
3. Incubate at 37 °C, 5% CO<sub>2</sub>.

## 7. Passaging (Day 3-4)

1. Passage is recommended just before or at the moment that cells reach confluence. Do not allow cells to become overconfluent, as this may lead to loss of endothelial phenotype.
2. Prepare fibronectin-coated vessels as previously described and allow them to equilibrate to room temperature before use.
3. Bring Accutase and Maintenance Medium to room temperature before use.
4. Aspirate the Maintenance Medium from the culture vessel.
5. Wash TrailBio® Endothelial Cells with DPBS (without Ca<sup>2+</sup> and Mg<sup>2+</sup>) using the volumes indicated in Table 7.
6. Aspirate the DPBS and add Accutase using the volumes indicated in the Table 7.
7. Incubate the culture vessel at 37 °C for 3 minutes.
 

**Note:** Confirm cell detachment under a microscope before diluting Accutase. If still adherent, incubate in 1-minute increments.
8. Dilute the Accutase by adding Maintenance Medium. Refer to Table 7 for the appropriate volumes.
9. Coat a pipette tip or serological pipette with Maintenance Medium, then gently pipette the TrailBio® Endothelial Cells up and down twice to wash the surface of the vessel and transfer the suspension to a 15 ml conical tube.
10. Wash the culture vessel with DPBS (without Ca<sup>2+</sup> and Mg<sup>2+</sup>) using the volumes indicated in Table 7 to collect any remaining cells.
11. Centrifuge the cell mixture for 5 minutes at 300 x g.
12. Aspirate the supernatant leaving behind ~100 µl to not disturb the cell pellet.
13. Resuspend the cell pellet in 2 ml of Maintenance Medium.
14. Remove a sample of the cell suspension and perform a cell count using a hemocytometer with trypan blue staining.
15. Add Maintenance Medium to the cells to achieve a target density of 10,000 cells/cm<sup>2</sup>.
16. Refer to Table 6 for seeding volumes and cell counts.
17. Aspirate fibronectin from the pre-coated culture vessel and immediately plate the cells.

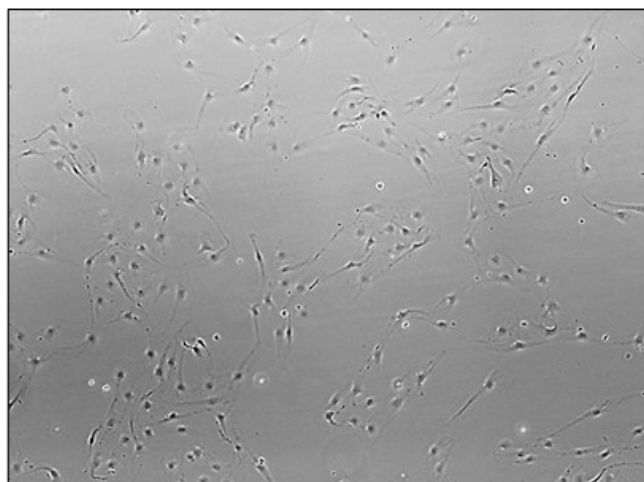
18. Gently move the vessel in a T-motion to ensure even distribution.

19. Incubate at 37 °C, 5% CO<sub>2</sub>.

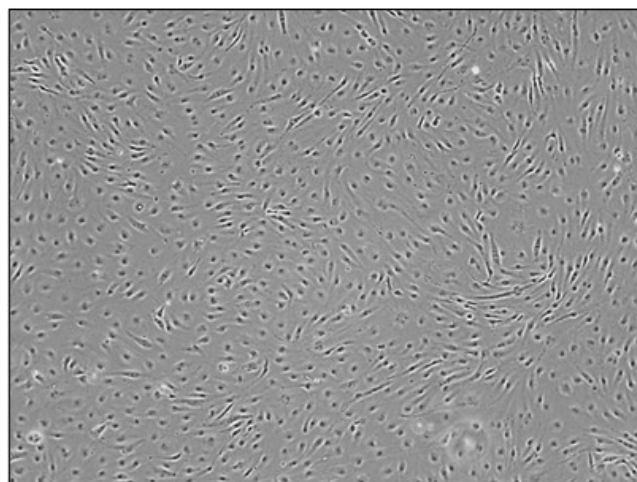
**Note:** It is not recommended to passage cells from a 96 well plate.

**Table 7: Accutase, Dilution, and Wash Volume Guidelines by Culture Vessel**

Plate Format	Accutase Volume	Dilution Volume	Wash Volume
T-25 flask	3 ml	3 ml	3 ml
6 well plate	1 ml	1 ml	1 ml
12 well plate	500 µl	1 ml	1 ml
24 well plate	250 µl	500 µl	500 µl



**TrailBio® Endothelial Cells 24hr post-thaw**



**TrailBio® Endothelial Cells 72hr post-thaw**

## 8. Expected Results

- **Days 0-1:** Small, phase-bright cells should be attached to the culture surface. A low seeding density and the presence of some non-viable floating cells are expected and will be removed during the first medium change.
- **Days 3-4:** Plates will begin to approach confluency, at which point passaging is required.

## 9. Troubleshooting Guide

Observation	Potential Cause(s)	Recommended Solution
<p><b>Low cell viability after thaw (&lt;80%)</b></p>	<ol style="list-style-type: none"> <li>1. Improper storage (e.g., at -70°C).</li> <li>2. Slow thawing process.</li> <li>3. Over-centrifugation.</li> <li>4. Improper handling of cells.</li> <li>5. Disturbing cell pellet after centrifugation.</li> </ol>	<ol style="list-style-type: none"> <li>1. Confirm cells are stored in LN2 vapor phase only.</li> <li>2. Work quickly to remove DMSO as fast as possible.</li> <li>3. Ensure centrifuge speed is 300 x g.</li> <li>4. Handle cells gently. Excessive pipetting can lead to shear stress.</li> <li>5. Leave ~100 µl of liquid above the pellet after centrifugation.</li> </ol>
<p><b>Cells are detaching / peeling from plate</b></p>	<ol style="list-style-type: none"> <li>1. Incomplete or dried-out plate coating.</li> <li>2. Media changes are performed too aggressively.</li> <li>3. Wrong plates used for culture.</li> </ol>	<ol style="list-style-type: none"> <li>1. Ensure coated plates are sealed and used within the specified time.</li> <li>2. Aspirate media very gently from a corner of the well.</li> <li>3. Use tissue culture plates to ensure cell adherence.</li> </ol>
<p><b>Culture appears sparse / low density</b></p>	<ol style="list-style-type: none"> <li>1. Inaccurate initial cell count.</li> <li>2. Loss of cells during plating/aspiration steps.</li> </ol>	<ol style="list-style-type: none"> <li>1. Ensure accurate cell counting with trypan blue exclusion.</li> <li>2. Be gentle during all aspiration and media change steps to avoid dislodging adhered cells.</li> </ol>

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