TrailBio® Endothelial Expansion Medium User Instructions

CONTENTS

1. Product Description 2
2. Before You Begin: Critical Success Factors 3
3. TrailBio® Endothelial Expansion Medium Preparation 3
4. Thaw Cells and Plating 3
5. Using the Medium 3
6. Troubleshooting Guide 4

1. Product Description

TrailBio® Endothelial Expansion Medium is a two-component kit consisting of TrailBio® Basal Medium F and TrailBio® Endothelial Expansion Supplement. Combined as directed, they form a complete medium that supports the attachment, survival, and proliferation of both **primary human endothelial cells (e.g. HUVECs)** and **iPSC-derived cells** (pair with **TrailBio® Endothelial Cells** for guaranteed performance), in culture.

This document covers the preparation, storage, and use of TrailBio® Endothelial Expansion Medium only. Matrix selection and coating, culture vessel format, thawing, seeding density, passaging reagents, and expected morphology are determined based on the cell line in use. Always follow the cell manufacturer's instructions for those steps.

For the expansion of iPSC-derived endothelial cells, please download the user instructions from the TrailBio® Endothelial Cells webpage or click here.

Table 1: Medium Kit components (TB99709):

Component Name	Size	Quantity	Storage	Catalog Number
TrailBio® Basal Medium F	180 ml	1 bottle	2-8 °C, Shipped on ice	TB73279
TrailBio® Endothelial Expansion Supplement	60 ml	1 bottle	-20 °C, Shipped on dry ice	TB03489

Notes

- *Store components individually at the recommended storage conditions. Use medium and supplements within 6 months from date of manufacturing.*
- *For use with **primary endothelial cells and iPSC-derived cells**, per the cell manufacturer's culture instructions.*

Table 2: Division of responsibilities between these instructions and your cell manufacturer

Follow these instructions for	Follow cell manufacturer's instructions for
Medium and supplement storage	Matrix / coating reagent and coating protocol
Preparing complete medium	Culture vessel format and surface area
Complete medium shelf life and handling	Thawing and cryopreservation
Medium-related troubleshooting	Seeding density and passage ratio
	Dissociation reagent and passaging method
	Expected morphology, markers, and passage limits
	Feeding volumes/schedules

2. Before You Begin: Critical Success Factors

This product is guaranteed to perform as described only when the following instructions are followed precisely. Failure to adhere to these steps can lead to suboptimal results.

Initial Checklist:

1. Confirm that the medium and supplement bottles are undamaged and that the supplement is frozen upon receipt.
2. Immediately transfer TrailBio® Basal Medium F to 2-8 °C and TrailBio® Endothelial Expansion Supplement to -20 °C upon receipt. Do not refreeze thawed supplement or subject it to repeated freeze-thaw cycles, as this can result in a loss of performance.
3. Read this entire protocol before starting any experimental work.
4. Use the matrix, culture vessels, and supporting reagents specified by your cell manufacturer.
5. Use proper sterile cell culture techniques throughout.

3. TrailBio® Endothelial Expansion Medium Preparation

1. Thaw TrailBio® Endothelial Expansion Supplement at 2-8 ºC overnight or at room temperature (15 - 25 ºC). Mix thoroughly. Do not warm to 37 ºC.
2. Add 60 ml of TrailBio® Endothelial Expansion Supplement to 180 ml of TrailBio® Basal Medium F and mix thoroughly.
   ***Note**: Prepared 'complete' medium can be stored at 2* °C *to 8* °C *for up to 14 days, protected from light.*

4. Thaw Cells and Plating

1. Add the appropriate volume of complete medium to newly thawed or passaged cells according to the cell manufacturer's recommended culture procedure.

   ***Note:** Data published on the website was generated using medium supplemented with Y-27632 for the first 48 hrs after thaw and passage steps. Follow cell manufacturer's protocol for further guidance.*

5. Using the Medium

1. Bring the required volume of complete medium to room temperature (15 - 25 °C) before use. Do not warm it to 37 °C.
2. Perform a full medium change every 48 hours, or at the frequency specified by your cell manufacturer, using the volume appropriate for your culture vessel.
3. Aspirate spent medium gently from the side of the vessel to avoid dislodging attached cells and add fresh medium slowly against the vessel wall.
4. If cell manufacturer specifies an additive such as Y-27632 at thaw or passage, add it to the complete medium immediately before use.
5. Return complete medium to 2-8 °C immediately after use and keep it protected from light.

6. Troubleshooting Guide

Observation	Potential Cause(s)	Recommended Solution
Precipitate or cloudiness in freshly prepared complete medium	1. Supplement not fully thawed before addition. 2. Supplement or basal medium warmed above room temperature. 3. Components combined without adequate mixing.	1. Thaw supplement at 2-8 °C overnight or at room temperature. Confirm it is fully thawed before use. 2. Never warm supplement or medium to 37 °C. 3. Mix thoroughly but gently after combining; avoid vigorous shaking.
Poor attachment or slow proliferation despite correct cell handling	1. Supplement subjected to repeated freeze-thaw cycles. 2. Medium over-warmed before use. 3. Matrix or coating not prepared per the cell manufacturer's instructions. 4. Complete medium used beyond 14 days.	1. Prepare fresh complete medium and record the preparation date on the bottle. 2. Do not repeatedly freeze thaw the supplement. 3. Equilibrate only to room temperature before use. 4. Confirm coating reagent, concentration, and incubation time against the cell manufacturer's protocol.
Rapid color change in the medium between scheduled feeds	1. Feeding interval too long for the culture density. 2. Cells seeded above the density recommended by the cell manufacturer. 3. Microbial contamination.	1. Perform a full medium change every 48 hours, or more frequently for dense cultures. 2. Seed at the density specified by the cell manufacturer. 3. Inspect under magnification and discard affected cultures and medium.
Cells detach or peel during medium changes	1. Aspiration or dispensing directed onto the growth surface. 2. Cold medium added directly to the culture.	1. Aspirate gently from the corner or side of the vessel and dispense slowly against the wall. 2. Bring medium to room temperature before feeding.

Also available from Trailhead Biosystems®

Cells

- TrailBio® Endothelial Cells – Compatible with TrailBio® Endothelial Expansion Medium
- TrailBio® Hematopoietic Progenitor Cells

Small Molecules

- TrailBio® Y-27632 dihydrochloride (Cat No. TB37849)
- TrailBio® Cytarabine (Ara-C) (Cat No. TB47622)
- TrailBio® SB-431542 (Cat No. TB46416)
- TrailBio® LDN-193189 dihydrochloride (Cat No. TB86388)
- TrailBio® CHIR-99021 monohydrochloride (Cat No. TB54925)

  For a full catalog of our small molecules, visit www.TrailBio.com/small-molecules

Services

- TrailBio® Custom iPSC Differentiation

For details of the above cells and services, visit www.trailbio.com

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