TrailBio® Hematopoietic Progenitor Cells User Instructions

Product Description

This protocol is designed to instruct users on how to thaw TrailBio® Hematopoietic Progenitor Cells.

Component (vial of 1M viable cells):

Component Name	Size	Quantity	Storage	Catalog Number
TrailBio® Hematopoietic Progenitor Cells	1 ml	1 vial	LN2, Shipped on Dry Ice	ME060001020

Note: *Use cells within 6 months of date of purchase.*

Materials required but not included:

Name	Vendor	Catalog Number
DPBS with Ca^{2+} and Mg^{2+}	Various	Various
Bovine serum albumin	Various	Various
Ultra-low adhesion cell culture plates	Various	Various
Sterile 15 ml centrifuge tubes	Various	Various

Note: *Materials listed here are required for the thawing protocol and are not supplied with the cells. Additional reagents and consumables such as cytokines and specialized culture plates may be needed for downstream applications but are not included in this protocol or provided with the cells.*

Cell Thawing and Preparation Protocol:

Note: *Use proper sterile culture techniques throughout all coating, preparation and culture steps. Do not remove cells from liquid nitrogen storage until you are ready to thaw. Once removed, proceed immediately to thaw.*

Plating medium and consumable preparation:

1. Warm desired plating medium in a 37 °C water bath.

Note: *Coat all tubes and pipette tips with 5% BSA in DPBS (with Ca^{2+} and Mg^{2+}) prior to cell contact to minimize cell loss.*

2. To coat tubes, add 5 ml of the 5% BSA solution into a new 15 ml conical tube and vortex briefly to coat the tube.
3. Once coated, remove and discard the 5% BSA solution, leaving ~150 µl in the tube.
4. The remaining 5% BSA can be used to coat all tubes and tips prior to cell contact.

Thawing cells:

Note: *It is recommended to thaw only one frozen vial at a time to prevent prolonged exposure of DMSO at higher temperatures. It is important to work quickly in the following steps to ensure high cell viability and recovery.*

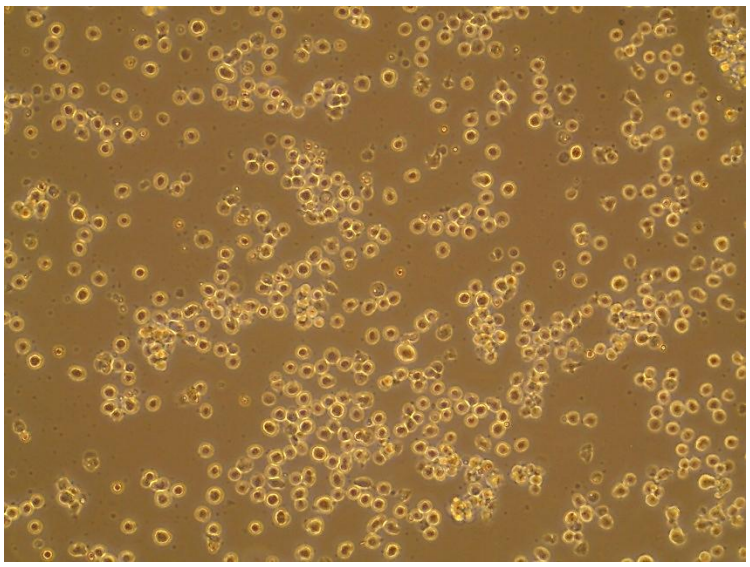
1. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
2. Quickly transfer the vial into the biosafety cabinet, slightly loosen the cap (quarter-turn) to release internal pressure, then retighten.
3. Swirl the vial in a 37 °C water bath until the contents are mostly thawed, leaving a small ice pellet.

Note: *Do not vortex cells.*

4. Transfer cell suspension to a 15 ml conical tube coated with 5% BSA using a 1 ml wide-bore pipette tip without mixing.

Note: *Ensure pipette tips are pre-coated with 5% BSA before handling cells*

5. Rinse the vial with 1 ml of pre-warmed plating medium and add it dropwise to the cells, while gently swirling the 15 ml tube.
6. Rinse the vial again with another 1 ml of pre-warmed plating medium adding it dropwise to the cells gently swirling the 15 ml tube.
7. Slowly add 12 ml of pre-warmed plating medium dropwise to the cells while continuously swirling the 15 ml tube.
8. Gently invert the 15 ml tube twice to mix.
9. Centrifuge the cell suspension at 350 x g for 4 minutes at room temperature (15 – 25 °C).
10. Carefully remove the supernatant with a pipette, leaving a small amount of plating medium to ensure the cell pellet is not disturbed.
11. Resuspend the cell pellet by gently flicking the tube.
12. Resuspend the cells in 1 ml of pre-warmed plating medium.
13. Take a sample of the cell mixture to perform a cell count. Each vial should contain $\geq$ 1 million viable cells determined by trypan blue staining.



TrailBio® Hematopoietic Progenitor Cells 1 day post-thaw

Next Steps:

Cells are ready for downstream applications. Seeding density and media composition may vary based on the experimental protocol. Ultra-low adhesion plates are recommended for lineage differentiation. For guidance, refer to your application-specific instructions or contact TrailBio technical support.