# TrailBio® Vascular Leptomeningeal Cell Kit User Instructions

## Product Description

This protocol is designed to generate functional vascular leptomeningeal cells (VLMCs) from a frozen vial of immature VLMCs following 7 days of differentiation.

### Reagents included in kit (KEC04000301; kit with vial of 3M cells):

| Component Name | Size | Quantity | Storage | Catalog Number |
|---|---|---|---|---|
| VLMC: Vascular Leptomeningeal Cells | 1 ml | 1 vial | LN2, Shipped on Dry Ice | EC4-007 |
| TrailBio® Basal Medium A | 200 ml | 1 bottle | 4 °C | BPS01020 |
| TrailBio® Plating Supplement C | 1 ml | 1 vial | -20 °C | PPS03100 |
| TrailBio® Vascular Leptomeningeal Cell Medium Supplement | 2.5 ml | 2 vials | -20 °C | DEC04400 |

### Materials required but not included:

| Name | Vendor | Catalog Number |
|---|---|---|
| Vitronectin recombinant human protein, truncated | ThermoFisher Scientific | A14700 |
| DPBS without Ca and Mg | ThermoFisher Scientific | 14190-144 |
| Accutase™ | STEMCELL Technologies | 07922 |
| Cell culture plate | Various | Various |
| Sterile 50 ml Tubes | Various | Various |
| 15 ml Centrifuge tubes | Various | Various |

***Note:*** *Trailhead Biosystems® has no affiliation with, nor is it endorsed by, any other vendors.*

## Preparation of Reagents:

***Note:*** *Use proper sterile culture techniques throughout all coating, preparation and culture steps.*

**Coating of culture plate:**

Follow manufacturers' instructions for coating culture vessels. Briefly, see below:

1. Thaw vitronectin at room temperature.
2. To make coating solution, dilute vitronectin in DPBS to reach 5 µg/ml concentration.
3. Distribute the solution evenly in each well (i.e. 50 µl in a 96-well plate).
4. Parafilm wrap the culture vessel and incubate it overnight at 4 °C.
5. Bring the culture vessel to room temperature prior to use for cell culture.
6. Remove the remaining coating solution completely, move quickly to avoid wells drying.

**Plating Medium preparation:**

1. Thaw a vial of Plating Supplement C at room temperature or overnight at 4 °C.
2. Add Plating Supplement C to Basal Medium A. Pipette up and down 2-3 times avoiding bubble formation, to ensure it is thoroughly mixed.
    - Add 975 µl of Plating Supplement C to 14.03 ml of Basal Medium A.

***Note:*** *Plating Medium must be used on the day of preparation and should be at room temperature prior to use. Freeze/thaw cycles of prepared Plating Medium are not recommended. Repeated freeze/thaw cycles of medium and supplements are not recommended.*

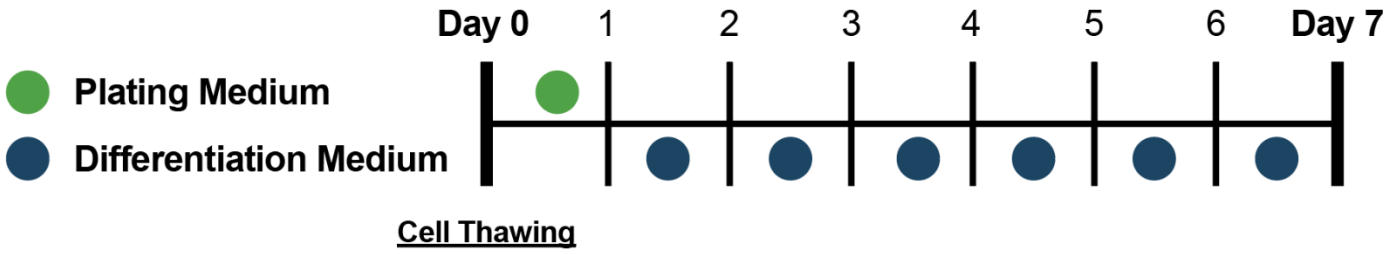
**Differentiation Medium preparation*:***

1. Thaw a vial of Vascular Leptomeningeal Cell Medium Supplement at room temperature or overnight at 4 °C.
2. Add Vascular Leptomeningeal Cell Medium Supplement to Basal Medium A. Pipette up and down 2-3 times avoiding bubble formation, to ensure it is thoroughly mixed.
    - Add 2.475 ml of Vascular Leptomeningeal Cell Medium Supplement to 42.525 ml of Basal Medium A.

***Note:*** *Prepared Differentiation Medium can be stored at 4 °C up to a week and should be at room temperature prior to use. Freeze/thaw cycles of prepared Differentiation Medium are not recommended. Repeated freeze/thaw cycles of medium and supplements are not recommended.*

## Directions for Use:

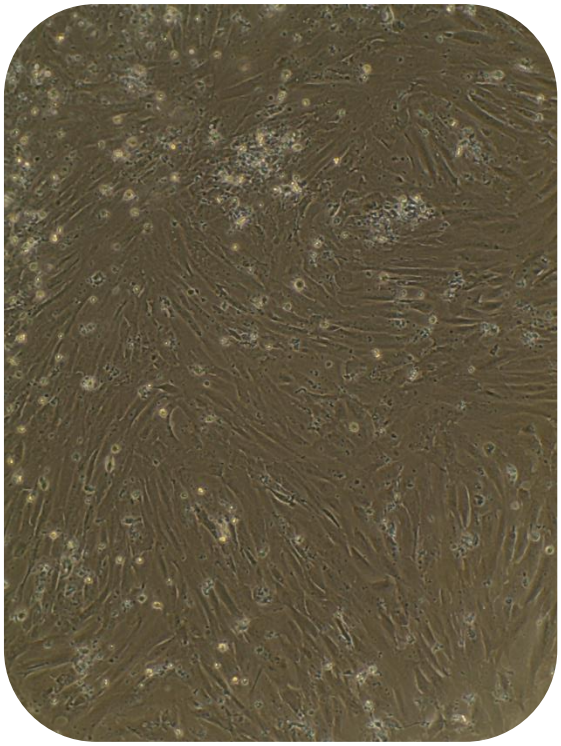
**Summary of Differentiation Protocol:**




**Thawing Frozen Cells (Day 0):**

***Note:*** *The addition of Plating Supplement C to Basal Medium A results in the preparation of Plating Medium and will be referred to as such, going forward.*

1. Prepare Plating Medium as per the "Plating Medium Preparation" step above.
2. Swirl the vial of cryopreserved cells in 37 °C water bath for two minutes until the cell mixture is nearly but not completely thawed, leaving a small ice pellet in the vial.
3. Transfer content of vial to a centrifuge tube and dilute the cell mixture with 5 ml of Basal Medium A.
4. Centrifuge the cell mixture for 4 minutes at 200 g.
5. Aspirate the supernatant gently and resuspend the cell pellet in 2 ml of Plating Medium.
6. Take a sample of the cell mixture to perform a cell count. Each vial should contain $\geq$ 3 million viable cells.
7. Adjust Plating Medium volume to the cells to achieve a target density of 50K cells/cm$^2$.
8. Plate cells onto coated plates (see above) and incubate at 37 °C.



Cells on day 7 post-thaw

**Differentiation (Day 1, onward):**

***Note:*** *The addition of Vascular Leptomeningeal Cell Medium Supplement to Basal Medium A results in the preparation of Differentiation Medium and will be referred to as such, going forward.*

1. Prepare Differentiation Medium as per the “Differentiation Medium Preparation” step on Page 2.
2. Gently aspirate the Plating Medium from the wells, rinse gently with DPBS, and add Differentiation Medium. For a 96-well plate, use 100 µl per well.

***Note:*** *Non-viable cells are expected in the culture and will be removed during media exchange.*

3. Change medium every 24 hours for a total of 6 days, preparing additional Differentiation Medium as needed.

***Note:*** *40 ml of Differentiation Medium should be sufficient for 3 daily medium changes per cell culture plate. i.e. 1 ml per well of a 12-well plate or 100 µl per well of a 96-well plate.*

4. On Day 7, cells may be used for downstream assays such as coculture experimentation.