

A Division of CA3 Biosciences, Inc.

## Harvesting Endothelial Cells from BBB model

- 1. Remove old media from the top of the inserts
- 2. Gently rinse inside of the inserts with 0 .5ml of Barrier wash solution.
- 3. Remove Barrier wash solution from the inserts.
- 4. Add 0.5ml of Cell detachment factor to the inserts.
- 5. Place the inserts with the plate in a 37 degrees Celsius incubator with 5% CO2 for 2-3 minutes.
- 6. Remove plate with inserts from the incubator and with a 0.5ml pipette, pipette up and down to help loosen the cells. (If cells do not come off, repeat step 5).
- 7. Transfer the cells into 10ml tube, add 3.5ml AlphaStop into the 10ml tube, and centrifuge at 1,500 RPM for 10 minutes.
- 8. Discard AlphaStop solution and keep cell pellet.



