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 a 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.