Title

Preparation of transwell inserts

Protocol

Materials:

- EBM-2 Medium (Cat. No.: CC-3156, Lonza)
- Phosphate Buffered Saline (PBS) (Catalog Nr: D8537, Sigma Aldrich, Co., stored at 2-8°C)
- Collagen type I coated 24-well plate transwell inserts
- Ethanol

Procedure:

To recreate the apical and basolateral sides present at the blood-brain barrier in vivo, cells can be seeded in transwell inserts of various sizes. In this sop, transwell inserts that fit a 24-well plate are detailed. When transferring the transwell inserts into the 24-well plate for coating and subsequent cell seeding, avoid direct contact with the inserts to minimize the risk of contamination. Instead, use forceps to handle the inserts. Following the appropriate coating of the transwell inserts as outlined in the SOPs, cells can be seeded in transwell inserts placed in a 24-well plate.

1. Start by adding 980 µl of medium to the basolateral chamber.
2. Following the cell detachment and counting procedures detailed in the SOPs, aspirate the old medium and resuspend the cell pellet in an appropriate volume of medium to achieve the desired cell concentration for 240 µl.
3. Carefully seed the cells onto the upper side of the transwell inserts. The volumes chosen for the basolateral and apical sides are based on those used to measure cell permeability and tightness in the CellZscope or during the performance of permeability assays.