Determination of Apical to Basolateral Permeability for 21-Day Caco-2 Cell Monolayers for Falcon® 96 Multiwell Insert Systems

Protocol

CORNING

Purpose

This is a method to determine the apical to basolateral permeability of [14C]-mannitol in cell monolayers grown on Falcon 96 Multiwell Inserts using a manual (non-robotic) procedure.

Materials

- Falcon 96 Multiwell Insert System [1.0 µm, 0.0804 cm^2 membrane surface area] (Cat. Nos. 351130 and 351131 includes feeder tray, Cat. No. 353938 includes receiver plate)
- Receiver Plates:
  - Falcon 96 Square Well, Angled-Bottom Plates (Cat. No. 353925)
- Wash Buffer:
  - Hanks Balanced Salt Solution (HBSS) +10 mM HEPES, pH 7.4
- Receiver Solution:
  - HBSS +10 mM HEPES +0.5% DMSO
- Donor Solution:
  - 50 µM [14C or 3H]-Mannitol in HBSS +10 mM HEPES +0.5% DMSO

Example:

- 5 µL of [14C]-Mannitol stock (Amersham Cat. No. CFA238, 3.4 mM, 0.2 µCi/µL)
- 44.9 µL of 10 mM Mannitol (non-radioactive) in DMSO
- 5.1 µL of DMSO
- 9935 µL HBSS +10 mM HEPES

Procedure

Washing the Monolayers

1. Transfer the insert plate from the feeder tray or 96 well receiver plate to an empty 96 well receiver plate.
2. Wash the monolayers, one column at a time:
   - Using a yellow tip attached to a vacuum, aspirate the culture medium from the apical side of a column of monolayers.
   - Using a multichannel or repeating pipettor, add back 50 µL of wash buffer to the apical sides of the monolayers.
3. Place the insert plate into a feeder tray containing 30 mL of wash buffer, or a 96 well receiver plate containing 260 µL of wash buffer/well. Keep plate at 37°C.

Permeability Assay

1. Transfer the insert plate from the wash plate to an empty 96 well receiver plate.
2. Remove the apical wash buffer and add the donor solution, one column at a time:
   - Using a yellow tip attached to a vacuum, aspirate the wash buffer from the apical side of a column of monolayers.
   - Using a multichannel or repeating pipettor, deliver 50 µL of donor solution to the apical sides of the monolayers.

3. Place the insert plate into a 96 well receiver plate containing 260 µL of receiver solution/well.
4. Incubate the plate at 37°C, with orbital shaking at 50-100 rpm for 2 hours.

After 2 hours of incubation:

5. Remove the insert plate from the receiver tray, place it into an empty 96 well receiver plate, and transfer 40 µL of the solution in the apical chamber (donor at t = 120 min.) to scintillation vials. Count.
6. Transfer 100 µL of the solution in the basolateral chamber (receiver at t = 120 min.) to scintillation vials. Count.
7. Count 50 µL of the original donor solution (t = 0 min.). This represents the amount of Mannitol added to the monolayers (donor). Determine pmol/dpm ratio.

   Example:

   52699 dpms counted in 50 µL of 50 µM (50 pmol/µL) mannitol donor (50 µL) x (50 pmol/µL) = 2500 pmol added to donor side 2500 pmol / 52699 dpms = 0.0474 pmol/dpm
8. Calculate amount of pmol in receiver tray. Dpms in 100 µL of receiver chamber solution x volume factor (2.6) = total dpms in chamber x pmol / dpm ratio = pmol in receiver chamber
9. Calculate Mannitol flux from donor side (apical) to receiver side (basolateral).

   Flux = pmol in receiver chamber at 120 min. / 2500 pmol in donor chamber at t = 0-10.
   Calculate Mannitol P_{app} at 120 min. (cm/sec).

   P_{app} = (Flux of drug) x (vol in donor chamber) / (sec of incubation) x (surface area)

   P_{app} = (Flux) x (0.05 cm^3 / [7200 sec x 0.0804 cm^2])
10. Calculate Mannitol mass balance (donor and receiver chambers).
    Mass Balance = ([pmol in 120 min. receiver + pmol in 120 min. donor] / 2500 pmol) x 100

Corning acquired the Falcon® brand.
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