

# Preparation of 21-Day Caco-2 Cell Monolayers for BD Falcon™ 96-Multiwell Insert Systems

## Protocol

### Purpose

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

### Materials

#### • Inserts:

– BD Falcon 96-Multiwell Insert System [1.0 µm, 0.0804 cm<sup>2</sup> membrane surface area] (Cat. Nos. 351130 and 351131 includes feeder tray, Cat. No. 353938 includes receiver plate)

#### • Receiver Plates:

– BD 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 min. Calculate Mannitol Papp at 120 min. (cm/sec).  

$$P_{app} = (\text{Flux of drug}) \times (\text{vol in donor chamber}) / (\text{sec of incubation}) \times (\text{surface area})$$

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