

# Protocol for Mannitol Permeability Measurement



- Materials:**
- FALCON® Companion Plates (Becton Dickinson Labware, Bedford, MA), 6-well—Cat. #353502, 12-well—Cat. #353503, 24-well—Cat. #353504
  - <sup>3</sup>H-Mannitol (NEN, New York, NY), Cat. #NEN101
  - PBS without Ca<sup>++</sup> Mg<sup>++</sup> buffer (Gibco/Life Technologies, Rockville, MD), Cat. #14190144
  - 0.05% Trypsin (Gibco/Life Technologies, Rockville, MD) Cat. #25300-062—0.53 M EDTA in HBSS without Ca<sup>++</sup> Mg<sup>++</sup> (can be frozen down 10 ml aliquots at -20°C)
  - DMEM, low glucose media with 1-glutamine, supplemented with 20% FBS (Gibco/Life Technologies, Rockville, MD), Cat. #11885-084
  - Transport Buffer (CellGro, St. Louis, MI), Cat. #21031—CM, supplemented with 1 mM CaCl<sub>2</sub>

1. Prepare <sup>3</sup>H-Mannitol in PBS by adding 1 µl <sup>3</sup>H-Mannitol stock (1 mCi) per 1 ml PBS. The following volumes will be needed per plate size: 6-well—12 mls, 12-well—18 mls, 24-well—22 mls. **NOTE:** PBS should be at room temperature. If <sup>3</sup>H-Mannitol was stored at 4°C, allow sufficient time to reach room temperature.
2. Add the appropriate volume of PBS to each Companion plate as follows: 6-well—2 mls per well, 12-well plates—1.5 mls per well, 24-well—1 ml per well.
3. Remove plate and inserts with Caco-2 cells from the incubator and discard growth media from the inserts. **NOTE:** We recommend gently dumping the inserts from the plate because pipetting the media can disrupt the monolayer.
4. Place inserts into the Companion plate containing PBS and add the appropriate volume of <sup>3</sup>H-Mannitol in PBS to the top of the insert as follows: 6-well—1 ml per insert, 12-well—.75 mls per insert, 24-well—0.31 mls per insert.
5. Allow the Companion plate with <sup>3</sup>H-Mannitol in PBS to incubate at room temperature for 30-60 minutes then discard the inserts into a radioactive waste disposal container.
6. Transfer 50 µl of the solution in the Companion plate (bottom chamber) into scintillation vials and then add 5 mls of scintillation fluid into each vial. Prepare a 50 µl aliquot of the starting <sup>3</sup>H-Mannitol solution as the standard. Place vials in a scintillation counter.

$$P_{app} = \frac{\text{Volume of Receptor Chamber (mls)}}{[\text{Area of Membrane (cm}^2\text{)}] [\text{Cpms/ml added}]} \frac{\Delta \text{ Cpms/ml}}{\Delta \text{ Time (sec)}}$$

This will give you a number for Mannitol Permeability. Typical units are x 10<sup>-6</sup> cm/sec.

If using compounds, modify the formula as follows: *Note*—in place of Cpm, use µM (starting concentration)

$$P_{app} = \frac{\textcircled{1} \text{ Volume of Receptor Chamber (mls)}}{\textcircled{2} [\text{Area of Membrane (cm}^2\text{)}] \textcircled{3} [\text{Initial Concentration}]} \frac{\textcircled{4} \Delta \text{ Concentration}}{\textcircled{5} \Delta \text{ in Time (sec)}}$$

- ① Volume of Receptor Chamber (mls) = 1 ml or cm<sup>3</sup>
- ② Area of Membrane (cm<sup>2</sup>) = 6-well—4.2 cm<sup>2</sup>, 12-well—1.75 cm<sup>2</sup>, 24-well—0.31 cm<sup>2</sup>
- ③ Initial Concentration = concentration of test compound or what is put into the inserts measured in µM or nM.
- ④ Δ Concentration = must be in concentration units or same concentration terms. If it is in nM, plug in nMolar (then LCMS is looking for nMOLES). To convert to concentration, divide the number of nMOLES by the volume of receptor chamber to give an appropriate concentration term that matches the starting concentration term.
- ⑤ Δ in Time (sec) = total incubation time converted to seconds.