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Protocol

EpiIntestinal™ Tissue Drug Permeation Protocol

For use with EpiIntestinal Tissue Models (SMI-100, SMI-100-FT, SMI-196, SMI-196-FT)

I. Buffers

a) **A-side transport buffer (Donor side buffer)**: 1.98 g/L glucose in 10 mM HEPES, 1x Hank's Balanced (Salt Solution) pH 6.5. The A-side buffer also contains **100 µM Lucifer yellow dye**.

b) **B-side transport buffer (Receiver side buffer)**: 1.98 g/L glucose in 10 mM HEPES, 1x Hank's Balanced (Salt Solution) pH 7.4

II. Drug Application Protocol for SMI-100, SMI-100-FT

a) **Apical to Basolateral (A → B) permeability:**

i) Add the required amount of drug to the apical donor side transport buffer to obtain the desired concentration (e.g. 10 µM).
ii) Apply 200 µL of the drug-containing buffer to the apical tissue surface.
iii) Add 500 µL of the B-side transport buffer to the basolateral/receiver side.
iv) Determine amount of permeation by collecting receiver buffer on the basolateral (B) side at time 2 hr.

b) **Basolateral to Apical (B → A) permeability:**

i) Add the required amount of drug to the basolateral (B) receiver side transport buffer to obtain the desired concentration (e.g. 10 µM).
ii) Place 500 µL of the drug-containing buffer on the basolateral side of the tissue.
iii) Add 200 µL of A-side buffer to the apical side.
iv) Determine the amount of permeation by collecting apical side buffer on the apical (A) side at time 2 hr.

III. Drug Application Protocol for SMI-196, SMI-196-FT

a) **Apical to Basolateral (A → B) permeability**:

i) Add the required amount of drug to the apical donor side transport buffer to obtain the desired concentration (e.g. 10 µM).
ii) Apply 75 µL of the drug-containing buffer to the apical tissue surface.
iii) Add 250 µL of the B-side transport buffer to the basolateral/receiver side.
iv) Determine the amount of permeation by collecting receiver buffer on the basolateral (B) side at time 2 hr.

b) **Basolateral to Apical (B → A) permeability:**

i) Add the required amount of drug to the basolateral (B) receiver side transport buffer to obtain the desired concentration (e.g. 10 µM).
ii) Place 250 µL of the drug-containing buffer on the basolateral side of the tissue.
iii) Add 75 µL of A-side transport buffer to the apical side.
iv) Determine the amount of permeation by collecting apical side buffer on the apical (A) side at time 2 hr.

IV. Permeability Analysis

Analyze the collected buffers using LC/MS/MS. Determine the permeability coefficient (*Papp*):

$P_{app} = \frac{dQ/dt}{C_0 A}$, where dQ/dt is the flux (amount of drug/ unit time), C_0 is the initial concentration of test and A is the area of the monolayer.

In the bidirectional permeability studies, the Efflux Ratio (R_E) is also calculated:

$R_E = \frac{P_{app}\ (B \rightarrow A)}{P_{app}\ (A \rightarrow B)}$. An $R_E > 2$ indicates a substrate which has active efflux transporters.

Note: To verify EpiIntestinal tissue membrane integrity at the end of the permeability experiment, aliquots of the cell buffers are analyzed by fluorescence to determine the transport of the impermeable dye Lucifer Yellow in the direction of A → B. Any deviations from control values should be recorded.