MatTek CORPORATION

Protocol

EpiAirway™ Drug Permeation Protocol

For use with EpiAirway Tissue Models (AIR-100 and AIR-100-SNP)

I. Receipt of EpiAirway Tissues

a) **Equilibration of EpiAirway tissues:** Upon receipt, the EpiAirway tissues need to be returned to culture for 18-24 hours. Under sterile conditions, pipet 0.9 ml (for AIR-100) or 2.0 ml (for AIR-100-SNP) of pre-warmed assay medium (AIR-100-ASY) in 6-well plates. Transfer the cell culture inserts containing the tissues into the 6-well plates and place the 6-well plates in the incubator (37ºC, 5% CO_2) overnight.

II. Permeability Experiments

a) **Receiver fluid preparation:** Pre-warm the EpiAirway assay medium (provided) to 37°C. Using sterile technique, pipet the medium into each well of the sterile 24-well plates (provided). Use the 24-well plates and 0.3 ml of medium per well for AIR-100; use 6-well plates and 2.0 ml of medium per well for the AIR-100-SNP. Other receiver fluids can also be used. Label the 24-well plates to accommodate 4 AIR-100 tissues measured at 6 time points or the 6-well plate to accommodate 1 AIR-100-SNP tissue at 6 time points. Label the wells as 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 hrs. Note: Additional assay time points which are more closely spaced or which extend to longer times may be necessary depending on how rapidly the drug permeates through the tissue.

b) **Donor solution:** If one is using a radio-labeled permeant, a donor solution of 2-3 µCi/ml is recommended. Use up to 0.2 ml of donor solution for both the AIR-100 and AIR-100-SNP tissues. For non-radio-labeled permeants, one needs to pick an appropriate donor concentration such that the analytical method will detect the permeant in the receiver solution. For example, depending on the drug, receiver solution concentrations may be 10-1000 fold below that of the donor solution. A sample of the donor solution and receiver solution (assay medium) must be saved for later analysis.

c) **Permeability experiment:** Following the overnight equilibration, move the cell culture inserts to the 0.5 hr wells and pipet the donor solution onto the tissue. Return the plates to the incubator. After 30 minutes of elapsed permeation time, move the tissues to 1 hour wells. Similarly move the tissues after 2.0, 3.0, 4.0 and 6.0 hrs of total elapsed time. It will not be necessary to replenish the donor solution. Alternatively, one can completely remove the receiver solution at the appropriate time and replace with fresh, pre-warmed receiver fluid.

d) **Tissue integrity:** After the final receiver sample has been collected, the permeation experiment is complete. Tissue integrity can be checked at this point by measuring transepithelial electrical resistance (TEER) or by adding an indicator dye such as Lucifer yellow or Sodium fluorescein.

e) **Additional sampling of donor solution:** After the final time point, an additional sample of the donor solution should be taken from the cell culture inserts to insure that the donor solution concentration remained constant throughout the experiment.

III. Data Analysis

a) **Determine flux versus time:** Assay all receiver and donor samples for drug concentration. Determine the flux (moles/cm^2/hr) over each permeation time interval, the average donor solution concentration, and the initial receiver solution concentration (background). Construct a plot of flux versus time. The surface area of different EpiAirway tissues is:

Tissue Model	Surface area
AIR-100	0.6 cm^2
AIR-100-SNP	1.0 cm^2
AIR-606-SNP	4.2 cm^2
AIR-100-PC6.5	0.33 cm^2
AIR-100-PC12	1.12 cm^2
AIR-196	0.12 cm^2

b) **Determine steady state, average flux:** At some point during the experiment, steady state should be achieved, i.e. the flux should become constant (± 20%). The average flux is computed by averaging the flux over all the time intervals once steady state has been reached.

c) **Calculation of permeability coefficient, kp:** The permeability coefficient, kp, as defined by Fick's law, can be calculated from the following equation:

$$k_p = (\text{average flux}) / (C_D . C_R)$$

where: **average flux** is measured in moles/cm^2/hr
C_R is the concentration of the drug in the receiver solution (moles/ml)
C_D is the concentration of the drug in the donor solution (moles/ml)
k_p is given in cm/hr.

IV. Materials Provided

EpiAirway (Part No. AIR-100)

Quantity	Description	Part No.
24	EpiAirway tissues	AIR-100
4	6-well plates (sterile)	TC-6WP
2	24 well plates (sterile)	TC-24WP
100 ml	PBS rinse solution	TC-PBS
125 ml	Assay medium	AIR-100-ASY
10 ml	0.3% Triton X-100 solution	TC-TRI

EpiAirway (Part No. AIR-100-SNP)

Quantity	Description	Part No.
24	EpiAirway tissues	AIR-100-SNP
6	6-well plates (sterile)	TC-6WP
100 ml	PBS rinse solution	TC-PBS
125 ml	Assay medium	AIR-100-ASY
10 ml	0.3% Triton X-100 solution	TC-TRI

V. Optional Materials

Quantity	Description	Part No.
1	Uncoated Millicell	MILCEL-CM
1	ECM Coated Millicell	MILCEL-ECM
1	Uncoated Snapwell	SNP-WEL
1	ECM Coated Snapwell	SNP-ECM
250 ml	Maintenance Medium	AIR-100-MM