MatTek LIFE SCIENCES

Protocol

EpiAirway™ Drug Permeation Protocol

I. Receipt of EpiAirway Tissues

Equilibration of EpiAirway tissues: Upon receipt, the EpiAirway tissues need to be returned to culture for 18-24 hours. Under sterile conditions, pipet the indicated volume of pre-warmed assay medium into the specified plate, according to Table 1. Transfer the cell culture inserts containing the tissues into the multi-well plates and place in the incubator (37ºC, 5% CO_2) overnight.

Tissue Format	Surface Area (cm^2)	Receiver Plate	Media Volume (mL / well)
AIR-100	0.6	24-well	1.0
AIR-100-PC6.5/PE6.5	0.33	24-well	0.5
AIR-100-PC12/PE12	1.12	12-well	0.75
AIR-100-SNP	1.12	6-well	2.0
AIR-606	4.2	6-well	2.0
AIR-196-HTS	0.12	96-well	0.25

Table 1. Equilibration of EpiAirway Tissues.

II. Permeability Experiments

Receiver fluid preparation: Pre-warm the EpiAirway assay medium (provided) to 37°C. Using sterile technique, pipet the suggested volume of medium into each well of the sterile multi-well plates (provided), according to Table 2. Other receiver fluids and volumes can also be used. Label the multi-well plates to accommodate up to four replicate tissues measured at six time points: 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.

Donor solution: Use up to the suggested volumes of donor solution for each tissue insert, according to Table 2. If using a radio-labeled permeant, a donor solution of 2-3 µCi/mL is recommended. 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.

Permeability experiment: Following the overnight equilibration in Part I, 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.

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.

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.

Tissue Format	Receiver Plate	Receiver Medium Volume (mL / well)	Donor Solution Volume (mL / tissue)
AIR-100	24-well	0.3	0.2
AIR-100-PC6.5/PE6.5	24-well	0.3	0.11
AIR-100-PC12/PE12	12-well	0.56	0.2
AIR-100-SNP	6-well	2.0	0.2
AIR-606	6-well	2.0	1.4
AIR-196	96-well	0.1	0.04

Table 2. Insert Formats & Suggested Volumes.

III. Data Analysis

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 given in Table 1.

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.

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. Optional Materials

Quantity	Description	Part No.
1	Uncoated insert (for AIR-100)	MILCEL-CM
1	ECM Coated insert (for AIR-100)	MILCEL-ECM
1	Uncoated Snapwell (for AIR-100-SNP)	MILCEL-SNP
1	ECM Coated Snapwell (for AIR-100-SNP)	MILCEL-SNP-ECM
1	Maintenance Medium, 250 mL	AIR-100-MM
1	Assay Medium, 125 mL	AIR-100-ASY
1	TEER buffer (PBS with Mg2+, Ca2+), 100 mL	TEER-BUFFER
1	PBS rinse solution (without Mg^{2+}, Ca^{2+}), 100 mL	TC-PBS