MatTek CORPORATION

Protocol

DRUG ABSORPTION PROTOCOL

For use with EpiOral™ Tissue Model (ORL-212-PERC & ORL-606-PERC)

I. Storage of EpiOral Tissues

a) **Storage:** The EpiOral tissue models are shipped on Monday for delivery on Tuesday morning. Upon receipt of the EpiOral Tissue Model, place the sealed plate containing the EpiOral tissue model samples and the assay medium into the refrigerator (2-8°C). *Note: EpiOral should be used within 48 hours of receipt. To improve inter-lot reproducibility, it is recommended that all experiments using a standardized protocol (e.g. all experiments commencing on Wednesday morning).*

II. Permeability Experiments

a) **Receiver fluid preparation:** Pre-warm the EpiOral assay medium (provided) to 37°C. Using sterile technique, pipet the medium into each well of the sterile 24-well or 6-well plates (provided). Pipet 0.3 ml/well of medium into the 24-well plates for the ORL-212 tissues; pipet 0.9 ml/well of medium into the 6-well plates for the ORL-606. If EpiOral assay medium will not be used as the receiver fluid, pipet the assay medium into 4 wells of the 24 well plate (or 1 well of the 6 well plate) and pipet the alternative receiver fluid into the remaining wells.

b) **Label plates with permeation times:** Label the 24-well plates to accommodate 4 tissues measured at 6 time points (or the 6-well plates to accommodate 1 tissue at 6 time points). Label the first well "1 hr equilibration." Label the remaining wells as 0.5, 1.0, 1.5, 2.0, and 2.5 hrs. *Note: i) This method involves moving the tissues from well to well at the appropriate time points and may require additional 24-well or 6-well plates. An alternative method is to remove all receiver solution at the appropriate time point (receiver solution is saved for later analysis) and re-fill the well with fresh receiver solution. ii) For high permeability drugs, shorter permeation time points such as 1, 5, and 15 minutes may be necessary.*

c) **Equilibration of EpiOral tissues:** Remove the EpiOral samples from the refrigerator. Under sterile conditions, transfer the EpiOral samples into the 1 hr equilibration wells containing the pre-warmed assay medium. Place the 24-well or 6-well plates in a 37ºC, 5% CO_2 incubator for 1 hour. After 1 hour, a baseline transepithelial electrical resistance (TEER) measurement (optional) can be made to insure barrier integrity. TEER values of >100 Ohm*cm^2 should be obtained.

d) **Donor solution:** If one is using a radio-labeled permeant, a donor solution of 2-3 µCu/ml is recommended. Use 0.4 ml of donor solution for ORL-200 tissues and 3.5 ml for the ORL-606 tissues. For non-radio-labeled permeants, one needs to pick an appropriately donor concentration such that the analytical method will detect the permeant in the receiver solution. For example, depending on the drug, receiver solution concentrations can 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.

e) **Permeability experiment:** Following the 1 hour 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 1.5, 2.0, and 2.5 hrs of total elapsed time. It will not be necessary to replenish the donor solution. Alternatively, after the 1 hour equilibration, remove the medium and replace with fresh, pre-warmed receiver solution. After 30 minutes of elapsed permeation time, remove the receiver solution and store in a tube appropriately labeled tube. Add fresh, pre-warmed receiver solution. At 1 hr total elapsed permeation time, remove the receiver solution, save it, and replace with fresh, pre-warmed receiver solution… etc.

f) **Tissue integrity:** After 2.5 hr of total permeation time, the permeation experiment is complete. Tissue integrity can be checked at this point by measuring TEER or by adding an indicator dye such as Lucifer yellow.

g) **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). The tissue area for the ORL-200 and ORL-606 tissue is 0.6 cm^2 and 4.2 cm^2, respectively. Construct a plot of flux versus time.

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, k_p:** The permeability coefficient, k_p, 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

EpiOral™ (Part No. ORL-212-PERC)

Quantity	Description	Part No.
12	EpiOral tissues	ORL-200
6	24-well plates (sterile)	MW-15-003-0028
50 ml	Assay medium	ORL-200-ASY
1	EpiOral Drug Absorption Protocol	MK-24-007-0014

EpiOral™ (Part No. ORL-606-PERC)

Quantity	Description	Part No.
6	EpiOral tissue-model samples	ORL-606
6	6-well plates	MW-15-003-0027
50 ml	EpiOral assay medium	ORL-200-ASY
1	EpiOral Drug Absorption Protocol	MK-24-007-0014

V. Optional Materials

Quantity	Description	Part No.
250 ml	Maintenance medium	ORL-200-MM
1	Uncoated cell culture inserts	MILCEL-MTK, MILCEL-606
1	ECM coated inserts	MILCEL-ECM-MTK, MILCEL-ECM-606