

DRUG ABSORPTION PROTOCOL

For use with EpiOral™ Tissue Model (ORL-212-PERC, ORL-200-PERC, ORL-606)

I. Storage of EpiOral Tissues

a) **Storage:** The EpiOral tissue models are shipped on Monday for delivery on Tuesday (US) or Wednesday (Europe & Japan). 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 72 hours from the time of shipment. 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) **Equilibration of EpiOral tissues:** Remove the EpiOral samples from the refrigerator. Pre-warm ORL-200-ASY to 37°C, add 0.3 mL/well to the 24-well (ORL-212-PERC) or 0.9 mL/well to the 6-well plates (ORL-606) provided. Under sterile conditions, transfer the EpiOral samples into the plates, confirm that the medium is in complete contact with the insert membrane, and place them in a 37°C, 5% CO₂ 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*cm² should be obtained.

b) **Choice of receiver fluid:** The EpiOral assay medium (Part #: ORL-200-ASY) or PBS containing glucose (Part #: TEER-BUFFER-GLC) are recommended as receiver fluids. If using the ORL-200-ASY, add your drug into the assay medium and make sure that proteins and other additives in the medium do not interfere with your analytical technique.

c) **Permeability experiments:** The tissues can be removed from the culture inserts using a sharp scalpel and placed in a Franz chamber or Ussing chamber. Alternatively, drug can be added directly into the cell culture inserts and inserts can be moved from well to well (filled with receiver fluid) at specific time points as follows:

i) Pre-warm the receiver fluid to 37°C.

ii) Pipet 0.3 mL/well of the receiver fluid into the 24-well plates for the ORL-212 tissues or 0.9 mL/well into the 6-well plates for the ORL-606.

iii) 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. Recommended time points are: 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 hrs.

iv) Pipet 0.4 mL of donor solution onto the ORL-200 and 3.5 mL onto the ORL-606 tissues.

v) After applying the donor solution, transfer the inserts into the wells containing the receiver solution. Confirm that there is no air trapped under the insert. Return the plates to the incubator. At the chosen permeation times, move the tissues to wells for the next time point. It is not necessary to replenish the donor solution.

Notes:

i) *Depending on the number of permeation time points chosen, this method may require additional 24-well or 6-well plates. An alternative method is to remove all receiver solution at the appropriate time point and re-fill the well with fresh, pre-warmed receiver solution. The receiver solution samples are saved for later analysis.*

ii) *For high permeability drugs, shorter permeation time points such as 1, 5, and 10 minutes may be necessary.*

d) **Donor solution:** If using a radio-labeled drug/permeant, a donor solution of 2-3 µCi/mL is recommended. For non-radio-labeled drugs, 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 can be 10-1000 fold below that of the donor solution. A sample of the donor solution and receiver solution must be saved for later analysis.

e) **Tissue integrity:** After the final permeation time point, the tissue integrity can be checked by measuring TEER or by adding an indicator dye such as Lucifer yellow.

f) **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/cm²/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 cm² and 4.2 cm², 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 ($\pm 20\%$). 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/cm²/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)

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
12	EpiOral tissues	ORL-200
3	24-well plates (sterile)	MW-15-003-0028
50 mL	Assay medium	ORL-200-ASY
1	PBS rinse solution, 125 mL	TC-PBS
1	EpiOral Drug Absorption Protocol	MK-24-007-0014

EpiOral™ (Part No. ORL-606)

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
6	EpiOral tissue-model samples	ORL-606
6	6-well plates	MW-15-003-0027
50 mL	EpiOral assay medium	ORL-200-ASY
1	PBS rinse solution, 125 mL	TC-PBS
1	EpiOral Drug Absorption Protocol	MK-24-007-0014

V. Optional Materials

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
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
125 mL	Receiver fluid (PBS w/ Ca ⁺² , Mg ⁺² , and glucose)	TEER-BUFFER-GLC

EpiOral™ (Part No. ORL-200-PERC)

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
24	EpiOral tissues	ORL-200
6	24-well plates (sterile)	MW-15-003-0028
50 mL	Assay medium	ORL-200-ASY
1	PBS rinse solution, 125 mL	TC-PBS
1	EpiOral Drug Absorption Protocol	MK-24-007-0014