

DRUG ABSORPTION PROTOCOL

For use with EpiGingival[™] Tissue Model (GIN-112-PERC & GIN-606)

I. Storage of EpiGingival Tissues

a) **Storage:** The EpiGingival tissue models are shipped on Monday for delivery on Tuesday (US) or Wednesday (Europe & Japan). Upon receipt of the EpiGingival Tissue Model, place the sealed plate containing the EpiGingival tissue samples and the assay medium into the refrigerator (2-8°C). *Note: EpiGingival 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).*

Part #	Description	Conditions	Shelf Life
GIN-112-PERC	EpiGingival cultures	Refrigerate (2-8°C)	96 hours*
GIN-606	EpiGingival cultures	Refrigerate (2-8°C)	96 hours*
GIN-100-ASY	Assay Medium	Refrigerate (2-8°C)	7 days

Notes: *Refers to storage time @ 2-8°C in unopened package.

II. Permeability Experiments

a) Choice of receiver solution: The EpiGingival assay medium (Part #: GIN-100-ASY) or PBS containing glucose (Part #: TEER-BUFFER-GLC) are recommended as receiver solution. If using the GIN-100-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.

b) **Receiver fluid preparation:** Pre-warm the EpiGingival 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). Use the 24-well plates and 0.3 mL of medium per well for GIN-112-PERC; use the 6-well plates and 0.9 mL of medium for GIN-606. If EpiGingival 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. For GIN-112-PERC, label the 24-well plates to accommodate 4 tissues measured at 6 time points (or for GIN-606, label the 6-well plates to accommodate 1 tissue at 6 time points). Label the first well "1 hr equilibration." Label the remaining wells as 1.0, 2.0, 4.0, 6.0, and 8.0 hrs.

Notes: 1) This method involves moving the tissues from well to well at the appropriate time point. 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. 2) Depending on the nature of the permeant, longer permeation times (> 8.0 hr) may be necessary.

c) **Equilibration of EpiGingival tissues:** Remove the EpiGingival samples from the refrigerator. Under sterile conditions, transfer the EpiGingival samples into the 1 hr equilibration wells containing the pre-warmed assay medium. Place the 24-well or 6-well plates in a 37° C, 5% CO₂ incubator for 1 hour. After 1 hour, a baseline transepithelial electrical resistance (TEER) measurement can be made to insure barrier integrity. TEER values of >150 Ohm * cm² should be obtained. Alternatively, the tissues can be removed from the culture inserts using a sharp scalpel and placed in a Franz chamber or Ussing chamber.

d) **Donor solution:** If using a radio-labeled permeant, a donor solution of 2-3 μ Cu/mL is recommended. Use 0.5 mL of donor solution for GIN-112-PERC tissues and 3.5 mL for the GIN-606 tissues. For non-radio-labeled permeants, 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 (assay medium) must be saved for later analysis.

e) **Permeability experiment:** Following the 1 hour equilibration, move the cell culture inserts to the 1.0 hr wells and pipet the donor solution onto the tissue. Return the plates to the incubator. After 1.0 hr of elapsed permeation time, move the tissues to 2.0 hour wells. Similarly move the tissues after 4.0, 6.0, and 8.0 hrs of total elapsed time. It will not be necessary to replenish the donor solution. Alternatively, after the 1 hr equilibration, remove the medium and replace with fresh, pre-warmed receiver solution. After 1.0 hr of elapsed permeation time, remove the receiver solution and store in a tube appropriately labeled tube. Add fresh, pre-warmed receiver solution. At 2.0 hr total elapsed permeation time, remove the receiver solution, save it, and replace with fresh, pre-warmed receiver solution... etc.

f) **Tissue integrity:** After the permeation experiment is complete, tissue integrity can be checked by measuring TEER or by adding an indicator dye such as Lucifer yellow. Alternatively, the dye can be added to the donor solution and the barrier can be monitored for the duration of the permeation experiment.

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) **Donor solution:** 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 GIN-112-PERC and GIN-606 tissue is 0.6 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:

 $k_p = (average flux) / (C_D - C_R)$

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

EpiGingival[™] (Part No. GIN-112-PERC)

Quantity	Description	Part No.
12	EpiGingival [™] tissue-model samples	GIN-112-PERC
3	24-well plates	MW-15-003-0028
1	EpiGingival assay medium, 50 mL	GIN-100-ASY
1	PBS rinse solution, 125 mL	TC-PBS
1	EpiGingival Drug Absorption Protocol	MK-24-007-0022

EpiGingival[™] (Part No. GIN-606)

1	Part No.
I [™] tissue-model samples	GIN-606
es	MW-15-003-0027
l assay medium, 50 mL	GIN-100-ASY
solution, 125 mL	TC-PBS
I Drug Absorption Protocol	MK-24-007-0022
	I [™] tissue-model samples es I assay medium, 50 mL solution, 125 mL I Drug Absorption Protocol

V. **Optional Materials**

Quantity	Description	Part No.		
1	Uncoated cell culture inserts	MILCEL-MTK, MILCEL-606		
1	ECM coated inserts	MILCEL-ECM-MTK, MILCEL-ECM-606		
250 mL	Maintenance medium	GIN-100-MM		
125 mL	Receiver solution (PBS w/ Ca+2, Mg+2, and glucose)	TEER-BUFFER-GLC		