

PERCUTANEOUS ABSORPTION

For use with EpiDerm™ Skin Model (EPI-200-X, EPI-212-X, & EPI-606-X)

I. Storage of EpiDerm Tissues

a) **Storage:** The EPI-200-X, EPI-212-X, and EPI-606-X EpiDerm skin models are shipped on Thursdays or Mondays for delivery on Tuesday morning. Upon receipt of the EpiDerm Skin Model, place the sealed plate containing the EpiDerm skin model samples and the assay medium into the refrigerator (2-8°C). *Note: EpiDerm should be used as soon as possible.* Storage conditions are summarized in the following table.

<u>Part #</u>	<u>Description</u>	<u>Conditions</u>	<u>Shelf Life</u>
EPI-200-X	EpiDerm cultures	Refrigerate (2-8°C)	96 hours*
EPI-212-X	EpiDerm cultures	Refrigerate (2-8°C)	96 hours*
EPI-606-X	EpiDerm cultures	Refrigerate (2-8°C)	96 hours*
EPI-100-ASY-X	Assay Medium	Refrigerate (2-8°C)	28 days
EPI-100-LLMM-X	Maintenance Medium	Refrigerate (2-8°C)	28 days

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

II. Preparation of EpiDerm For Percutaneous Absorption Measurements

a) **Pre-warm media:** Pre-warm the MatTek assay medium (provided) to 37°C. Using sterile technique, pipet the volume of the medium listed in the table below into the appropriate vessel(s) listed below.

<u>Part #</u>	<u>Vessel</u>	<u>Volume</u>
EPI-200-X	6-well plate	0.9mL
EPI-212-X	6-well plate	0.9mL
EPI-606-X	100mm petri dishes	6.3mL

b) **Transfer EpiDerm samples:** Remove the package containing the tissue samples from the refrigerator. Under sterile conditions, open the package and using sterile forceps, remove the inserts containing the tissues from the agarose of the package while the package is still at refrigerator temperature. Transfer the inserts into the vessel(s) containing the pre-warmed assay medium. *Note: Removal of the inserts while cold will minimize the difficulty of separating the inserts from the agarose and will minimize the amount of agarose that adheres to the inserts.* Care should be taken to remove all agarose sticking to the outside of the cell culture inserts containing the tissue samples.

c) **Incubate:** Place the vessel(s) containing the EpiDerm tissue samples into a humidified 37°C, 5% CO₂ incubator for 1 hour prior to dosing. *Note: Some laboratories have found that an overnight incubation (“pre-equilibration”) is preferable, especially if cytokine release or gene expression will be measured.* This extended pre-equilibration step allows the tissues to more fully recover from the stress of shipping. In any case, a standardized pre-equilibration time should be used.

d) **Choice of permeation device and EpiDerm tissue:** Standard permeation devices such as Franz chambers, Bronaugh cells, side-by-side diffusion chambers, etc. or the MatTek Permeation Device (MPD), a reusable permeation device which directly accept the EPI-200-X and EPI-212-X tissues, can be used to approximate the permeability of a material through the skin. However, the orifice size of the device (*i.e. area of exposed tissue*) will determine which EpiDerm model can be used as follows:

<u>Orifice diameter</u>	<u>EpiDerm Model</u>	<u>Usable Tissue Diameter</u>
≤5 mm	EPI-200-X, EPI-212-X	8 mm
>5 mm, ≤18 mm	EPI-606-X	22 mm

With the exception of the MPD, it will be necessary to remove the tissue from the cell culture insert as next described. Note: For accurate permeability measurements, the permeant cannot simply be added to the cell culture insert since permeant bypass will occur at the sidewall / tissue interface.

e) **Removal of EpiDerm sample from cell culture insert:** Fold 3-4 paper towels in half and wet them with phosphate buffered saline (PBS). Place the cell culture insert which contains an EpiDerm tissue on top of the wetted towels with the feet on the bottom the cell culture insert pressed into the towel. The sample can be removed from the plastic part of the cell culture insert using a sharp knife or a dermal punch. An 8 mm can be used with the EPI-200-X and EPI-212-X tissues and up to a 22 mm punch can be used with the EPI-606-X. If a punch is used, place the punch inside the cell culture insert and firmly press down to cut a tissue disc from the cell culture insert. *Note: Often the dermal punch will not make a complete cut. If this is the case, a fine cuticle scissors or a scalpel can be used to sever the EpiDerm sample from the cell culture insert.* The resultant disc is then placed stratum corneum side up (membrane side down) into the permeation device, as would be done with normal skin punch biopsy samples.

f) Tissue removal notes of caution:

1. When using the dermal punch to cut the EpiDerm sample from the cell culture insert, press straight down on the punch but do not rotate the punch. Rotation of the punch may tear or disrupt the EpiDerm barrier.
2. It is preferable not to remove the EpiDerm sample from the underlying microporous membrane of the cell culture insert. The underlying membrane is a highly porous (0.45 µm pores), Teflon-based, chemically inert material which should not affect permeability. However, if desired the EpiDerm sample can be gently teased away from the membrane using a pointed tweezers.

g) **Use of MatTek permeation devices:** Reusable, plastic permeation devices are available from MatTek Corporation (Part No. EPI-FIX). If these devices are to be used, the cell culture insert, which contains the EpiDerm tissue, is inserted between the 2 pieces of the device and 3 screws/nuts are tightened to create a seal between the bottom rim of the device's inner annulus and stratum corneum. *Note: The screws should be snug tightened by hand and then further tightened an additional half turn with a pliers or Allen wrench. The screws should not be over-tightened.* Any tightening which will deform the plastic device is too tight and will compromise the seal.

h) **Re-use of MatTek permeation devices:** The MatTek permeation devices can be sterilized by soaking in 70% ethanol for 30 minutes. However, MatTek permeation devices CANNOT be autoclaved since autoclaving will result in deformation of the device.

III. Permeability Experiments

a) **Donor solution:** If one is using a radio-labeled permeant, a donor solution of 2-3 µCu/mL is recommended. Donor solution volumes of 0.4 mL for EPI-200-X and EPI-212-X tissues and the MatTek permeation devices are adequate; however, other permeation cells/chambers may require a larger donor volume. The donor volume should be of sufficient volume such that the donor concentration does not drop more than 5% over the course of the permeation experiment. For non-radiolabelled permeants, one needs to pick an appropriately donor concentration such that the analytical method will detect the permeant in the receiver solution. For example, typically, receiver solution concentrations are 1000 fold below that of the donor solution. A sample of the donor solution must be saved for later analysis.

b) **Receiver solution:** 5.0 mL of PBS, TEER-BUFFER-GLC or EpiDerm Maintenance Medium (EPI-100-LLMM-X) can be used as the receiver solution with the MatTek Permeation Device (other permeation cells/ chambers may require different volumes). PBS is a suitable receiver solution for experiments that are ≤ 6 hours in duration, although TEER-BUFFER-GLC may provide better results if viability is to be measured after permeation. For experiments > 6 hours in duration, EPI-100-LLM-X should be used as the receiver solution. The loaded MPD and 5.0 mL of solution conveniently fit in the well of a 6-well plate. If using EPI-100-LLMM-X, check that medium does not interfere with the analytical technique or the accurate assaying of the permeant. A sample of the receiver solution must be saved for later analysis. *Note: Care must be taken to dislodge any air bubbles trapped beneath the tissue since intimate receiver fluid/tissue contact is required to obtain accurate permeability measurements.*

c) **Negative controls:** A convenient negative control is 0.02 wt % sodium fluorescein. This highly colored, fluorescent material will not pass through EpiDerm provided that the permeation chamber is appropriately sealed and therefore serves as a useful negative control (to insure that there is no fluid bypass around the edges of the tissue). In addition, it is a good idea to check that the permeant is not binding to the extra-cellular matrix coating on the cell culture insert upon which EpiDerm is cultured. Tissue-less, coated inserts are available for this purpose (Part No. MILCEL-ECM-MTK and MILCEL-ECM-606).

d) **Equilibrate tissue:** Prior to placing the donor solution on the EpiDerm sample, the permeation device containing the tissue should be equilibrated to the permeation temperature for 15 minutes. After this period, pipette the donor solution onto the stratum corneum - this marks time 0.0 for the experiment. See **Figure 1**.

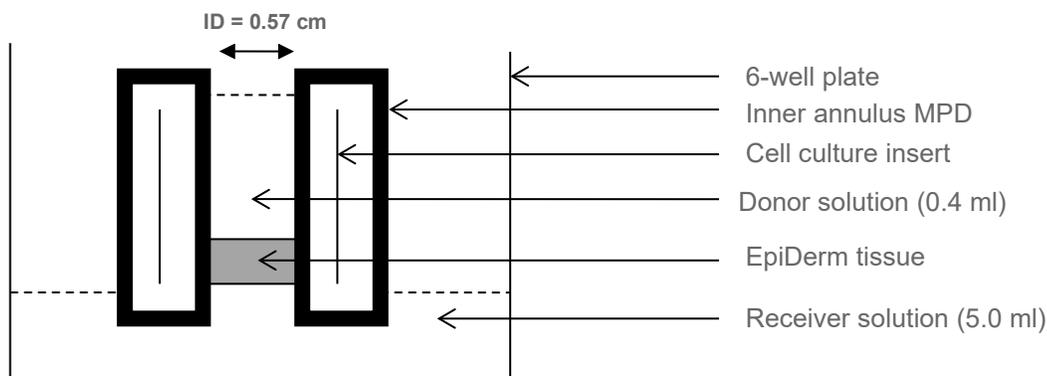


Figure 1: Permeability configuration utilizing MatTek Permeation Device (MPD) illustrating exposure of donor solution to center of EpiDerm tissue (EPI-200-X or EPI-212-X) thus preventing permeant bypass.

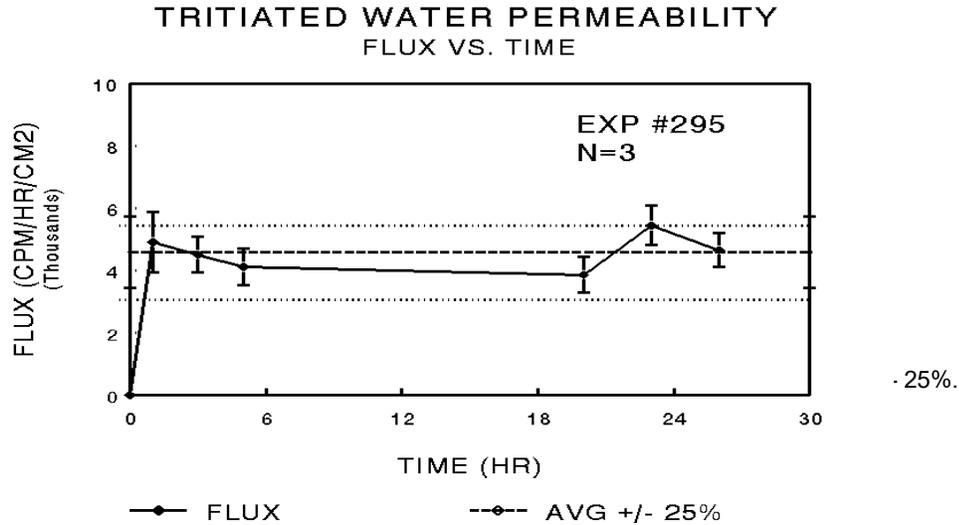
e) **Permeation times:** Most permeants will penetrate EpiDerm and reach steady permeation within 24 hours. Ideally, the more data points taken over this period the better, i.e. a flux measurement every 30 minutes or 1 hour. However, an accurate determination of the permeability coefficient, k_p , can often be made using the following time points: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 21.0, 21.5, 22.0, 23.0, 24.0, and 25.0 hrs. *Note: For highly permeable materials (i.e. $k_p > 10^{-2}$ cm/hr, more closely spaced time points ending at 5.0 hours may be necessary.)*

f) **Sampling receiver solution:** At each time point, the entire receiver solution should be removed, stored, and replaced with fresh receiver solution. If the MPD are used, place 5.0 mL of fresh receiver solution in another well of the 6 well plate and completely remove the 5.0 mL from the previous time point, saving the solution for later analysis. *Note: One needs to insure that the concentration of the permeant in the receiver solution is uniform.* A small magnetic stir bar can be used to mix the receiver solution during the permeability experiment.

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

h) **Prepare flux versus time plot:** Assay all samples for permeant 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 exposed area of tissue for the MPD is 0.256 cm². Construct a plot of flux versus time.

i) **Determination of 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. *Note: If the permeation times suggested above in e) are utilized, the overnight interval (between 5.0 and 21.0 hrs) will often show a different permeation value than the others.* If the overnight flux differs by more than 25% from the average, it should be ignored and not used in the calculation of the average steady state flux. A typical flux versus time plot is shown in **Figure 2**.



j) **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_D is the concentration of the drug in the donor solution (moles/mL)

C_R is the concentration of the drug in the receiver solution (moles/mL)

k_p is given in cm/hr.

IV. Materials Provided

EpiDerm (Part No. EPI-200-X)

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
24	EpiDerm skin-model samples	EPI-200-X
500 mL	PBS	TC-PBS
4	6 well plates	MW-15-003-0027
100 mL	Assay Medium (LLMM-X)	EPI-100-ASY-X

EpiDerm (Part No. EPI-212-X)

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
12	EpiDerm skin-model samples	EPI-200-X
250 mL	PBS	TC-PBS
2	6 well plates	MW-15-003-0027
50 mL	Assay Medium (LLMM-X)	EPI-100-ASY-X

EpiDerm (Part No. EPI-606-X)

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
6	EpiDerm skin-model samples	EPI-606-X
500 mL	PBS	TC-PBS
6	Petri Dishes 100 mm	MW-15-003-0031
100 mL	Assay Medium (LLMM-X)	EPI-100-ASY-X

V. Optional Materials

<u>Quantity</u>	<u>Description</u>	<u>Part No.</u>
1	Permeation Device	EPI-FIX
1	ECM-coated Cell Culture Inserts (min. order 6)	MILCEL-ECM-MTK**
1	ECM Coated Cell culture inserts (min. order 6)	MILCEL-ECM-606**
250 mL	Maintenance Medium (LLMM-X)	EPI-100-LLMM-X
125 mL	TEER Buffer (DPBS with Ca ²⁺ , Mg ²⁺ & Glucose)	TEER-BUFFER-GLC

** Use MILCEL-ECM-MTK with EPI-200-X and EPI-212-X. Use MILCEL-ECM-606 with EPI-606-X.