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Protocol

EpiIntestinal™ Small Intestine Full Thickness Tissue Model (SMI-100-FT) Use Protocol

I. Storage of Tissues (SMI-100-FT)

a) **Storage:** Upon receipt of the EpiIntestinal Tissue Model, place the sealed 24-well plate containing the EpiIntestinal tissues at room temperature (20-25°C) and the maintenance medium into the refrigerator (2-8°C). Transfer the tissues to the incubator as described in Section II, Preparation of EpiIntestinal, preferably on the day of arrival. Storage conditions for components of EpiIntestinal are summarized below:

Part #	Description	Storage Conditions	Shelf Life
SMI-100-FT	EpiIntestinal Tissues	Room Temperature	96 hours*
SMI-100-MM	Maintenance (apical) Medium	Refrigerate (2-8°C)	2 weeks
SMI-100-FT-MM	Maintenance (basal) Medium	Refrigerate (2-8°C)	2 weeks

Notes: **Refers to storage time at room temperature in unopened package from Monday at 3:00 PM (i.e. 96 hours would be Friday at 3:00 PM).*

Before performing any tests or experiments, the EpiIntestinal Tissue Model needs to be pre-equilibrated overnight. It is strongly recommended to proceed with Section II, Preparation of EpiIntestinal, immediately after receiving the tissue model.

II. Preparation of EpiIntestinal

a) **Prepare HNG-TOP-12 plate:** Per kit of 24 SMI-100-FT tissues, pre-warm 125 mL of SMI-100-FT-MM and 10 mL of SMI-100-MM in a 37°C water bath for 10 minutes. Under sterile conditions, open the HNG-TOP12 and remove together the regular lid and the hanging-top lid (**Figure 1A**) from the bottom of the 12-well plate (**Figure 1B**). Pipette 5.0 mL of the pre-warmed SMI-100-FT-MM medium into each well of the 12-well plate and replace the hanging-top lid on top of the bottom plate. Label the plates indicating the test material and the dosing time to be used**.**

b) Transfer tissues: Under sterile conditions, open the package containing the tissue samples and using sterile forceps, transfer the inserts from the agarose package into the hanging-top lid (**Figure 1C**). Care should be taken to remove all agarose sticking to the outside of the cell culture inserts containing the tissue samples. Pipette 200 µL of the pre-warmed SMI-100-MM medium (provided) onto the apical surface of each tissue (**Figure 2).**

c) Pre-equilibration: Place the regular lid over the hanging-top lid (**Figure 1D**) and return the fully assembled HNG-TOP-12 plate containing the EpiIntestinal samples into a humidified incubator overnight at 37°C, 5% CO_2. This pre-equilibration allows the tissues to recover from the stress of shipping (**Figure 2)**. *Note: Any air bubbles trapped underneath the cell culture insert should be released (tilt the cell culture insert using a sterile forceps) so that adequate nutrients are supplied to the EpiIntestinal tissues.*

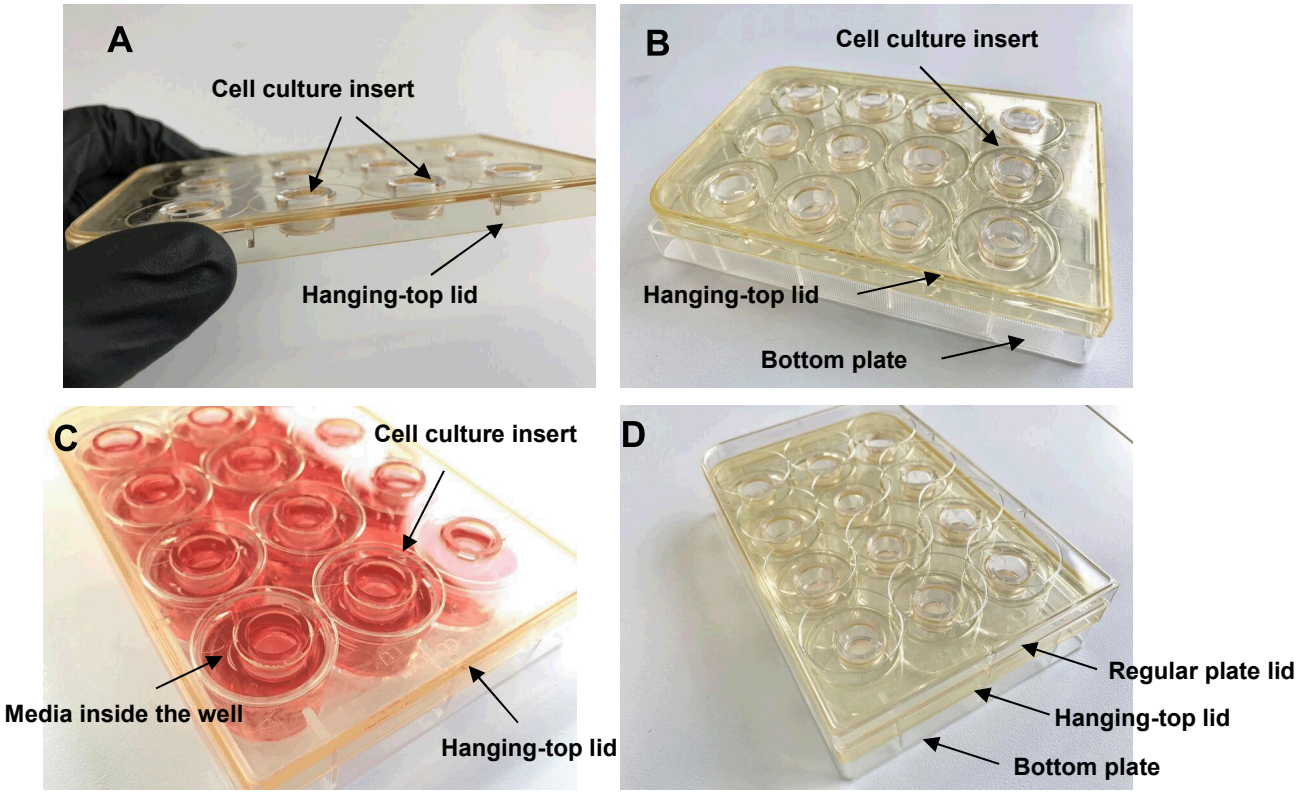

Figure 1: (**A**) Cell culture inserts (8.8 mm ID) in the hanging-top lid; (**B**) Hanging-top lid with inserts on top of the bottom plate without media; (**C**) Hanging-top lid on top of the bottom plate containing media; (**D**) Fully assembled HNG-TOP-12.

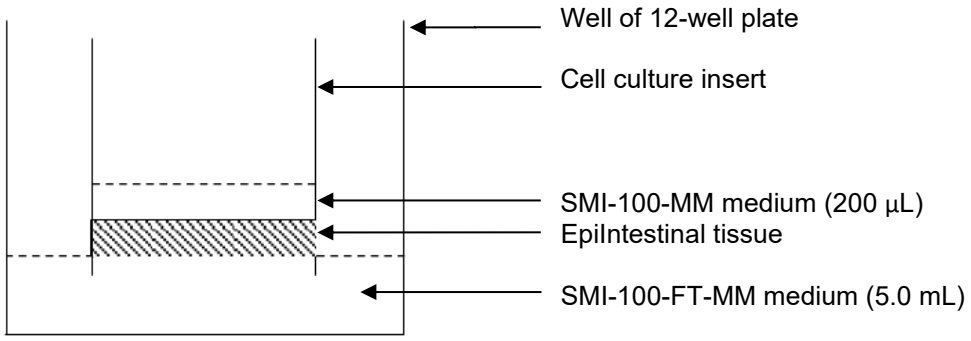

Figure 2: Pre-equilibration of EpiIntestinal tissue (overnight at 37°C, 95% rH, 5% CO_2). 200 µL of SMI-100-MM medium applied to apical tissue surface and 5.0 mL of SMI-100-FT-MM medium on the basolateral membrane surface

III. Dosing

a) **Replace medium:** Following the overnight incubation, aliquot 125 mL of SMI-100-FT-MM and 10 mL of SMI-100-MM (per kit of 24 SMI-100-FT tissues) using sterile technique. Pre-warm the aliquoted media for 10 min in a 37°C water bath. Aspirate off the SMI-100-FT-MM media contained in the bottom 12-well plates and replace with 5.0 mL (per well) of pre-warmed, fresh media. In addition, decant any SMI-100-MM remaining on the apical tissue surface. Note: Any air bubbles trapped underneath the cell culture insert should be released, as previously described. The tissues are now ready for dosing/experimentation.

b) **Treatment conditions:** To test the toxicity or efficacy of drug formulations or other test materials, apply the test article directly to the apical tissue surface using a positive displacement pipette. For short-term exposure (≤24 hrs), apply 50 µL of test material. For longer-term exposures (>24 hrs), apply 50 µL of test article and re-feed tissues with 5.0 mL of fresh SMI-100-FT-MM every other day (see next section and **Table 1**). For drug permeation studies, see Protocol # MK-24-007-0104. *Note: If DMSO is used to help solubilize the drug, the final DMSO concentration applied to the tissue should be ≤ 1.0%.*

Test materials (e.g. cytokines, growth factors, hormones, etc.) can also be added to basal compartment of the bottom 12-well plate containing the SMI-100-FT-MM to simulate systemic exposure to the tissue. Alternatively, bacteria, viruses, or other pathogens can be applied topically to the tissue surface (i.e. the intestinal lumen) to model intestinal infection. The surface area of the SMI-100-FT tissues is 0.6 cm^2 **(Figure 3).**

Note: Antibiotics (gentamicin) and antimycotics (Amphotericin-B) are utilized in the culture of the tissue model and are normally included in the maintenance media provided. However, tissues and media free of these agents are available upon request. Please contact a MatTek Technical representative to discuss your experimental requirements.

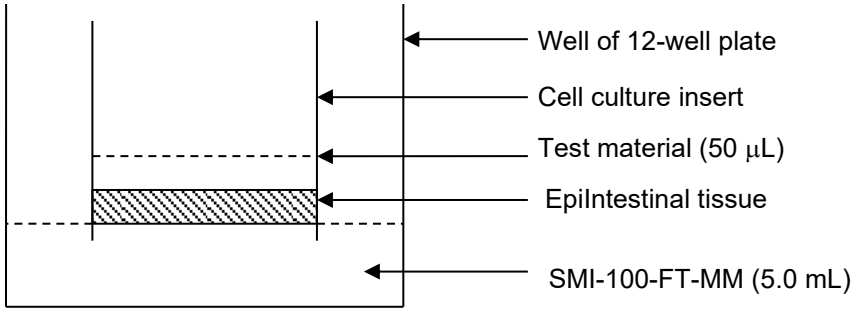

Figure 3: Dosing Configuration: 50 µL of test material applied to apical tissue surface and 5.0 mL of SMI-100-FT-MM medium on the basolateral membrane surface.

c) **Extended culture (>6 days):** Chronic exposure experiments with EpiIntestinal are possible provided that the SMI-100-FT-MM medium is replaced (every other day) with 5.0 mL of fresh, pre-warmed medium and the apical surface of the tissue is rinsed (every 7 days) as follows:

1. Aspirate the basolateral medium and add 5.0 mL of fresh, pre-warmed SMI-100-FT-MM into each well of the 12-well plates containing the tissue culture inserts.
2. If any medium remains on the apical surface, grasp the insert firmly with sterile forceps and decant the apical medium.
3. Topically apply 200 µL of the pre-warmed SMI-100-MM into each cell culture insert (onto the apical tissue surface).
4. Repeat steps 1-3 every Monday, Wednesday, Friday, and Saturday.
5. On Day 6 (and every 7 days thereafter), gently pipet 200 µL of PBS (pH 6.8) onto the apical tissue surface. Grasp the insert with sterile forceps and rock it back and forth and then decant. Repeat this procedure 2X to remove any cell debris sloughing off the apical tissue surface. Then proceed with medium change as described in steps 1–3. Tissues should be fed very Monday, Wednesday, Friday, and Saturday and the apical tissue surface should be rinsed every Monday **(Table 1)**.

d) **Inclusion of controls:** It is important to include negative, positive, and benchmark controls to compare the effects of the various test conditions. N=3 tissues are recommended for controls.

Day	Replace Media	Apical Rinse (PBS pH6.8)*	Note
0 (Tues)	+		Receipt of tissue, Post shipment recovery
1 (Wed)	+		Replace medium/apply dose
3 (Fri)	+		*
4 (Sat)	+		*
6 (Mon)	+	+	*
8 (Wed)	+		*
10 (Fri)	+		*
11 (Sat)	+		*
13 (Mon)	+	+	*
15….	…	….	Maintain culture as above

Table 1: Schedule for extended culture experiments using EpiIntestinal model.

*Notes:

a) The timing of test article re-application and analysis / endpoint measurements depend on the specific experiment design. Using proper sterile technique, TEER can be measured repeatedly on the same tissues (e.g. at 24 or 48-hour intervals). The apical medium, as well as any topically applied compounds, should be replenished after each TEER measurement.

b) Adjust the PBS rinse solution (provided) to pH 6.8. Alternatively, order part # DPBS-6.8.

e) **Choice of assay procedures:** Please contact your MatTek technical service representative for any additional protocols required. The effect of the test materials or treatments to the tissue can be monitored using a broad variety of endpoints and assay methods, including:

Barrier function: Use transepithelial electrical resistance (TEER) measurements to probe the status of tight junction within the tissue. The EVOM Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL) is recommended *(Protocol, MK-24-007-0084)*.

Gene expression: Place tissues in an RNA stabilization reagent (e.g. RNAlater), harvest RNA, and analyze gene expression using RT-PCR or q-PCR *(Protocol, MK-24-007-0065)*.

Protein expression: Place tissues in a lysis buffer, purify and quantify protein, and analyze using Western blot analysis *(Protocol # MK-24-007-0098)*.

Cytokine release: Save the apical or basolateral medium (beneath the tissue) and analyze secreted cytokines using commercially available ELISA or BioPlex kits.

Tissue viability: Measure the tissue viability using the MTT assay *(Protocol MK-24-007-0083)*.

Histology and Immunostaining: Fix, embed, section, and H&E stain the tissues to observe the tissue histology or perform immunohistochemical staining (IHC) to observe specific protein expression in the tissues *(Protocol OH-24-007-0001).*

Drug permeation: To model the permeability of drugs through the small intestine tissue, please request the EpiIntestinal Tissue Drug Permeation Protocol (*Protocol MK-24-007-0104).*

MatTek can also provide Contract Services to analyze the effects of your test materials.

IV. Materials Provided

EpiIntestinal™ (Part No. SMI-100-FT)

Quantity	Description	Part No.
24	EpiIntestinal tissues	SMI-100-FT
2	HNG-TOP-12 plates (sterile)	HNG-TOP-12
2	24-well plates (sterile)	MW-15-003-0028
1	PBS rinse solution, 100 mL	TC-PBS
1	Maintenance medium, 250 mL	SMI-100-FT-MM
1	Apical maintenance medium, 25 mL	SMI-100-MM
1	SMI-100-FT Use Protocol	MK-24-007-0131

V. Optional Materials

MTT Assay Kit (Part No. MTT-100)

Quantity	Description	Part No.
1	MTT diluent solution, 8 mL	MTT-100-DIL
1	Extractant solution, 60 mL	MTT-100-EXT
1	MTT concentrate (5:1), 2 mL	MTT-100-CON

Additional Materials (for extended culture times)

Maintenance Medium (Part No. SMI-100-FT-MM)

Quantity	Description	Part No.
1	Maintenance medium (for extended culture times), 250 mL	SMI-100-FT-MM
1 (included)	Apical maintenance medium, 25 mL	SMI-100-MM

Rinse Solution (Part No. DPBS-6.8)

1	PBS rinse solution, pH 6.8, 125 mL	DPBS-6.8

VI. Alternate Tissues

Quantity	Description	Part No.
24 tissues	SMI-100-FT cultured without antibiotics	SMI-100-FT-ABF
24 tissues	SMI-100 epithelial tissue	SMI-100
96 tissues	SMI-100-FT cultured in high throughput, 96-well plate	SMI-196-FT
96 tissues	SMI-100 cultured in high throughput, 96-well plate	SMI-196