Corning® BioCoat™ Intestinal Epithelium Differentiation Evironment

Catalog No. 355057

Lot No. 3312560

Instructions for Use

Use restriction for Europe and the United Kingdom – This product may only be used as an ***in-vitro*** laboratory reagent. This product and its residue must not be allowed to come into contact with ruminating animals or swine.

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For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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INTENDED USE

The Corning® BioCoat™ Intestinal Epithelium Differentiation Environment is designed promote the rapid differentiation of intestinal epithelium *in vitro.*

THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

SUMMARY

Investigation into the transport of compounds and infectious agents across the intestinal wall is of enormous importance in understanding of the structure and function of the intestinal epithelium and in the development of new pharmaceuticals. The ability to predict the bioavailability of orally administered compounds, or how orally administered compounds affect the intestinal uptake of various metabolites, can be a powerful tool to screen for potential new drug candidates. Heretofore, bioavailability data have been generated with the use of *in vivo* animal models. However, animal testing is elaborate and expensive and animal data cannot always be extrapolated to humans.

In search of a simpler, less expensive and more reliable alternative to animal models, *in vitro* model systems have been developed that use cells of human origin, such as the human colon adenocarcinoma-derived cell line Caco-2[1,2]. Among the drawbacks associated with *in vitro* systems are requirements for medium supplementation with undefined animal serum and for extended culture periods to obtain the differentiated phenotype of intestinal absorptive cells (enterocytes). In the case of Caco-2 cells, it has been reported that the establishment of mature barrier and transport functions in a serum-containing environment takes between two to four weeks[2,3].

These two concerns have been addressed by the development of the Corning BioCoat Intestinal Epithelium Differentiation Environment. Using this system, a differentiated enterocyte monolayer with barrier function can be established within two to three days in a serum-free environment. The system integrates Corning BioCoat Fibrillar Collagen Cell Culture Inserts (1 µm PET membranes with a thin layer of native type I collagen fibrils) and a fully defined, serum-free medium supplemented with butyric acid, hormones, growth factors, and other defined metabolites. Thus, the Corning BioCoat Intestinal Epithelium Differentiation Environment can be used to rapidly create an *in vitro* intestinal model for transport and adsorption studies of drugs and natural compounds, as well as for studies involving infectious agents in the intestine.

The Corning BioCoat Intestinal Epithelium Differentiation Environment contains:

- Seeding Basal Medium, Dulbecco's Modified Eagles Medium (DMEM) - 100 ml (Part No. 01-05450)
- Enterocyte Differentiation Medium - DMEM containing butyric acid-400 ml (Part No. 01-05448). Butyric acid appears to induce differentiation of intestinal epithelial cells *in vitro* (by down-regulating c-myc expression)[4].
- MITO+ Serum Extender- contains mouse EGF, human transferrin, bovine insulin, human recombinant ECGS, triiodothyronine, hydrocortisone, progesterone, testosterone, estradiol-17B, selenium and O-phophorylethanolamine)-lyophilized, 1 x 0.5 ml (Catalog No. 356007), each 0.5 ml vial will supplement 500 ml.

  NOTE: Any of the human source material used in the manufacturing of this material was tested and found nonreactive for hepatitis B surface antigen (HBsAG), for antibody to hepatitis C virus (anti-HCV), for antibody to human immunodeficiency virus-1 (anti-HIV-1) and for antibody to human immunodeficiency virus-2 (anti-HIV-2). Regardless of the test data this product should be handled observing the same Universal Safety Precautions employed when handling any potentially infectious material.

- Corning® BioCoat™ Fibrillar Collagen Cell Culture Inserts – Twenty four 6.4 mm inserts with type I collagen treated 1 μm pore size PET membrane (Catalog # 354474). The membranes are treated with rat tail collagen, type I, under conditions that allow *in situ* formation of large collagen fibrils with normal cross-striation pattern without the use of chemical cross-linking or ammonium vapor alkalinization. Inserts are packaged within a 24 well Falcon® Companion Tissue Culture Plate (Catalog # 353504).

- Falcon Companion Tissue Culture Plate -one additional 24-well plate provided for permeability studies (Catalog # 353504).

MATERIALS REQUIRED BUT NOT SUPPLIED

- Sterile deionized water
- Falcon sterile pipettes: 10 ml, 5 ml, 2 ml
- Falcon sterile centrifuge tubes: 50 ml
- Tissue culture hood
- Humidified tissue culture incubator, 37°C, 5% CO_2
- 0-200 µl and 200 - 1000 µl pipet device
- Sterile 0-200 µl and 200 - 1000 µl pipet tips
- Sterile forceps
- 70% ethanol
- Trypsin EDTA solution
- Caco-2 cells at 80% confluency (need 4.8 x 10^6 cells per insert plate with 24 inserts) **CAUTION**: Caco-2 cells at less than 80% confluency may result in unreliable barrier formation.
- Materials necessary for permeability studies: e.g., Phosphate Buffered Saline (PBS), compounds of interest

PRECAUTIONS

Storage:
Materials should be stored at 2-8°C in the original packaging with lid box closed to avoid possible deterioration of culture medium by light. **DO NOT FREEZE.**

Reconstitution:
Medium supplements should be added under aseptic conditions. Do not filter the supplemented medium. The components of this Corning BioCoat Intestinal Epithelium Differentiation Environment have been tested and found negative for the presence of bacteria, fungi and mycoplasma.

Use of Supplemented Medium:
The Seeding Basal Medium and Enterocyte Differentiation Medium must be supplemented with MITO+ Serum Extender prior to use. After supplementation both media are stable for 21 days when stored at 2-8°C in their original bottle. Prior to use, preincubate as much medium as necessary at 37°C in a separate container.

Reconstituted MITO + Serum Extender may be stored separately for up to 6 months at -20°C.

AVOID REPEATED WARMING/COOLING CYCLES OF THE STOCK OF SUPPLEMENTED MEDIUM, AS THIS MAY ADVERSELY AFFECT ITS BIOACTIVITY.

PROCEDURE FOR USE

ATTENTION: THIS ENVIRONMENT REQUIRES AN ATYPICAL MEDIA CHANGE SCHEDULE. PLEASE READ THESE INSTRUCTIONS CAREFULLY PRIOR TO USE.

NOTE: ALL PROCEDURES SHOULD BE PERFORMED UNDER STRICT ASEPTIC CONDITIONS. MEDIA AS SUPPLIED DOES NOT CONTAIN ANTIBIOTICS. ANTIBIOTICS CAN INTERFERE WITH PERMEABILITY OF SOME COMPOUNDS. IF THIS PRODUCT IS TO BE USED WITH PRIMARY CELL CULTURES THE ADDITION OF ANTIBIOTICS TO THE MEDIA IS RECOMMENDED.

1.0 Reconstitution of MITO+ Serum Extender and Supplementation of Media

1.1 Disinfect the exterior of the of MITO+ Serum Extender vial with 70% ethanol. Reconstitute the vial of MITO+ Serum Extender with 500 µl of sterile, deionized water (500 ul total).

1.2 Add 100 µl of MITO+ Serum Extender to Seeding Basal Medium (in the 125 ml bottle). Supplemented medium is stable at 2-8°C for up to 21 days.

1.3 Add 400 µl of MITO+ Serum Extender to 500 ml of Enterocyte Differentiation Medium. Supplemented medium is stable at 2-8°C for up to 21 days.

2.0 Cell Growth Phase: Seeding of Cells

2.1 For every insert to be seeded at this time, add 1 ml of prewarmed (37°C) supplemented Seeding Basal Medium to a well of an empty 24-well plate.

2.2 Using sterile forceps transfer the treated inserts into the wells which contain 1 ml of media (from step 2.1).

NOTE: If all of the treated inserts are not used at once, please use care to maintain the sterility of the unused inserts. Unused inserts should be stored at 2-8°C.

2.3 Determine the amount of supplemented Seeding Basal Medium required (0.5 ml x number of inserts to be seeded).

2.4 Add cells to the volume prewarmed of (37°C) supplemented Seeding Basal Medium determined in step 2.3 to achieve a cell density of 0.4 X 10^6 cells/ml.

NOTE: For best results cells should be at 80% confluency prior to seeding. Use of cells at lower confluency may result in unreliable measurements.

2.5 Add 0.5 ml of the cell suspension from step 2.4 to each insert processed in step 2.2. (This will result in a seed density of 200,000 cells per insert.)

2.6 Incubate at 37°C, 5% CO_2, and 100% humidity for 24 hours.

Making alterations to the 3 day time frame for Caco-2 Cells to accommodate working schedules:

Once the Enterocyte Differentiation Medium is added, the monolayers should be used after 48 hours (3 days "elapsed" time). By day 4, the monolayers may become leakier.

If some variability in the 3 day time is desired, some of the earlier steps in the growth of the monolayer may be adjusted to alter the schedule beyond 3 days. For example, the typical protocol calls for the following 2 steps:

Typical Protocol:

1) Day 1: Seed inserts with supplemented Seeding Basal Medium and wait 24 hours
2) Day 2: Change to supplemented Enterocyte Differentiation Medium and replenish 48 hours later as described in Section 3

Step 1 (first day growth) time frame of 24 hours is not too critical, so cells can be grown in the MITO+ Serum Extender - supplemented Seeding Basal Medium for up to 3 days, as long as the medium does not get depleted (pH shift) during the growth phase. It is, therefore, possible to start cell seeding on Thursday afternoon and change to supplemented Enterocyte Differentiation Medium on Friday morning. Replace medium with supplemented Enterocyte Differentiation Medium on Monday leading to monolayers 24 hours later on Tuesday. In some cases, medium maybe depleted before 3 days in which case media change would be required.. If desired, additional Seeding Basal Medium Enterocyte Differentiation Medium and MITO+ Serum Extender may be purchased separately as Catalog No. 355058.

3.0 Cell Differentiation Phase: Addition of Enterocyte Differentiation Medium

3.1 After completion of step 2.6, carefully remove the medium both from the plate well (basal aspect) and the interior of the insert (apical aspect).

CAUTION: Excessive aspiration force may cause the medium to pull the fibrillar collagen coating & cells off the membrane, resulting in erratic permeability measurements.

3.2 Add 1 ml of prewarmed (37°C) supplemented Enterocyte Differentiation Medium (Step 3.1) to the plate well of the insert and 500 µl to the interior of the insert.

3.3 Incubate at 37°C, 5% CO_2, 100% humidity. The time required for complete differentiation from this point is dependent on cell type. When differentiation is monitored by mannitol diffusion, the adenocarcinoma cell line Caco-2 is ready for testing in 48 hrs., and the rat enterocyte line IEC-18 is ready in 24 hrs.

3.4 Monolayers may be maintained by replacing spent media with fresh supplemented Enterocyte Differentiation Medium every 24 hours (Steps 3.2 - 3.4). For best results, we recommend using differentiated monolayers 48 hours after addition of supplemented Enterocyte Differentiation Medium.

Creating longer lasting monolayers

If longer lasting monolayers are desired, the following modifications to the protocol will prolong the life of the monolayer by several days. The modification calls for diluting the Enterocyte Differentiation Medium 1:10 with additional Seeding Basal Medium (Catalog No. 355257) and using the resulting solution to achieve differentiation. **(Both products should still be supplemented with MITO+ Serum Extender prior to use).** The advantage of this modification is that it provides a longer lasting monolayer. The disadvantage of this modification is that some differentiation marker & transport function enzyme activities (e.g., alkaline phosphatase) may not be "upregulated" to the same extent as the typical protocol. Sometimes it is also possible to maintain monolayers in supplemented Enterocyte Differentiation Medium by changing the medium every 24 hours (See Step 3.4). We suggest that either protocol be validated by the user with known samples prior to use.

RECOMMENDED PROTOCOL FOR PERMEABILITY STUDIES

4.0 Apical/Basal Permeability

4.1 Add 1 ml of Phosphate Buffered Saline (PBS) per well to an empty Falcon® Companion Tissue Culture 24-well plate (Catalog #353504).

4.2 Using sterile forceps, remove the insert from the supplemented Enterocyte Differentiation Medium, tipping the insert to pour the medium off the interior of the insert (apical side).

4.3 Place the insert in the PBS-containing well (from Step 4.1).

4.4 Gently add 310 µl of test material to the inside of the insert (apical side).

NOTE: Addition of a larger volume (> 310 µl) may create a hydrostatic head pressure on the cell monolayer. This pressure could introduce a pressure artifact into the permeability data obtained. If use of a larger volume is required, the user should ensure that the increased pressure is not introducing artifact into the results.

4.5 Incubate at room temperature. The length of this incubation time should be experimentally determined by each user for their particular compounds; however, in order to maintain "Infinite dose" kinetics an incubation period of 1 hour is recommended if the permeability coefficient of the compound is estimated to be < 10 $X10^{-6}$ cm/sec and 10 minutes if the permeability coefficient is estimated to be > 70 x 10^{-6} cm/sec.

4.6 Using forceps, shift the insert to a well containing fresh PBS.

4.7 Determine the concentration of test material in the PBS on the plate well side (ablumenal).

5.0 Basal/Apical Permeability

5.1 Add 1 ml of Phosphate Buffered Saline (PBS) containing the test material to the wells of an empty Falcon Companion Tissue Culture 24-well plate (Catalog #353504).

5.2 Using sterile forceps, remove the insert from the supplemented Enterocyte Differentiation Medium, tipping the insert to pour the medium off the interior of the insert (apical side).

5.3 Place the insert in the well containing the test material and PBS. (from Step 5.1)

5.4 Add 300 µl of PBS to the inside the insert (apical side).

5.5 Incubate at room temperature for a predetermined period of time (see step 4.5) and then remove 200 µl of PBS from the interior of the insert (apical side) to determine the concentration of test material. Replace the removed PBS immediately with 200 µl of fresh PBS.

TYPICAL RESULTS

The Corning® BioCoat™ Intestinal Epithelium Differentiation Environment has been tested with cells of human and rat origin: Caco-2 cells, a human colon adenocarcinoma cell line, and IEC-18 cells, a rat ileum (small intestine) epithelial cell line. The system will provide the following results when used according to instructions.

With Caco-2 cells:

a. Confluent monolayer of cells observed within 24 hours of seeding, starting with a seed density of 2×10^5 cells on a 6.4 mm insert (24-well size), in supplemented Seeding Basal Medium.

b. Establishment of barrier function as measured by transepithelial electrical resistance (TEER) within 72 hours of cell seeding or 48 hours after adding supplemented Enterocyte Differentiation Medium (with TEER of > 150 Ω-cm^2, see Figure 1, page 9).

c. Establishment of barrier function as measured by mannitol, rifampin and d-cycloserine permeabilities within 72 hours of cell seeding or 48 hours after adding supplemented Enterocyte Differentiation Medium (see Figure 2, page 9).

With IEC-18 cells:

a. Confluent monolayer of cells observed within 24 hours of seeding, starting with a seed density of 2×10^5 cells on a 6.4 mm insert, in supplemented Seeding Basal Medium.

b. Establishment of barrier function as measured by transepithelial electrical resistance (TEER) within 48 hours of cell seeding or 24 hours after adding supplemented Enterocyte Differentiation Medium (with TEER of 28 Ω-cm^2).

c. Establishment of barrier function as measured by mannitol permeability within 48 hours of cell seeding or 24 hours after adding supplemented Enterocyte Differentiation Medium (with mannitol permeability coefficient of < 7×10^{-6} cm/sec).

STABILITY

The components of this Corning BioCoat Intestinal Epithelium Differentiation Environment are stable for a minimum of 3 months from day of shipment when stored at 2-8°C. Upon supplementation with MITO+ Serum Extender, both the Seeding Basal and Enterocyte Differentiation Medium are stable for 21 days when stored at 2-8°C under subdued lighting conditions.

ORDERING INFORMATION

To place an order in the U.S., contact Customer Service at:
tel: 800.492.1110, fax: 978-442-2476; email: CLSCustServ@Corning.com

Outside the U.S., contact your local distributor or visit:
www.Corning.com/lifesciences to locate your nearest Corning office.

TECHNICAL SERVICES

For technical assistance, contact Technical Support at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSTechServ@Corning.com

REFERENCES

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[2]Rubas, W. et al., Pharm. Res. **10:**113 (1993)
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[4]Souleimani, A and Asselin, C., FEBS Letters **326:**45 (1993)

Figure 1

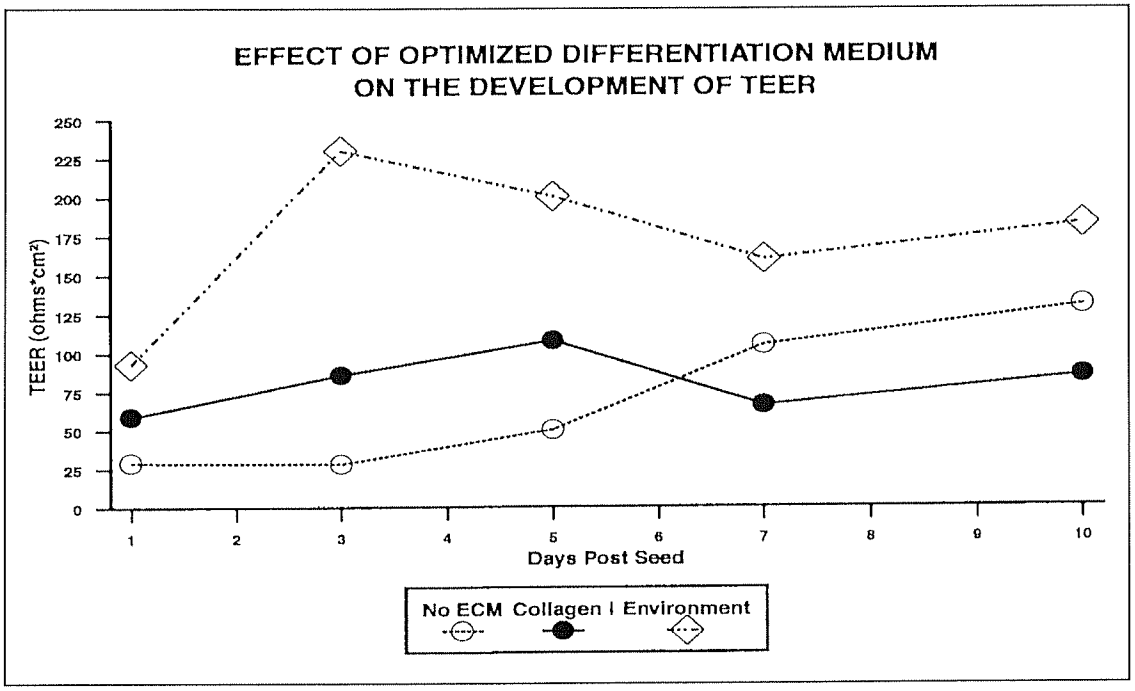
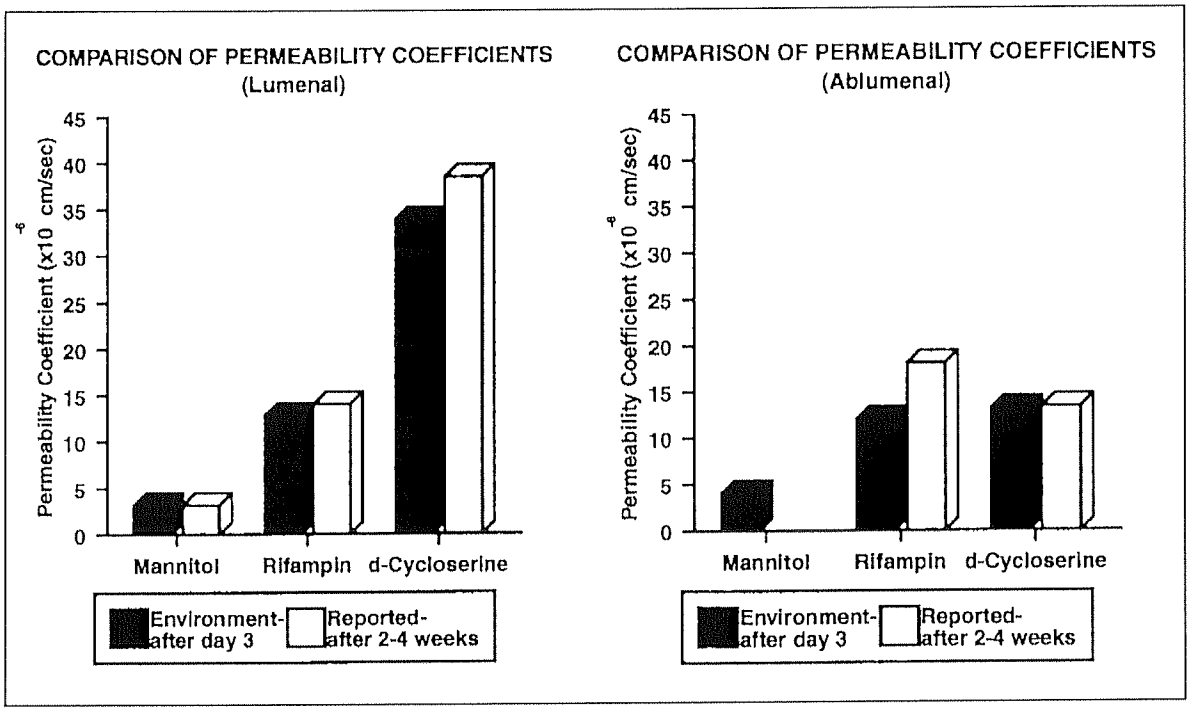


Figure 2



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