RepliGut® Planar Kit
Frequently Asked Questions (FAQs)

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scientificsupport@altisbiosystems.com

2 KIT COMPONENT STORAGE:

2.1 WHAT ARE THE STORAGE CONDITIONS AND THE SHELF-LIFE OF THE MEDIA AND KIT COMPONENTS?

See individual components for exact expiration dates. General expiration guidelines are in the table below.

Reagent	Storage Condition	Expiration*
RepliGut® Planar Precoated Transwell® Plate	4°C	4 weeks
RepliGut® Growth Medium (RGM)	4°C	1 month
RepliGut® Maturation Medium (RMM)	4°C	1 month
Cell Dissociation Solution***	4°C	1 month
Sterile 1× Phosphate Buffered Saline (PBS)	Room Temperature	Lot Specific
Culture Plate Sealing Tape**	Room Temperature	N/A
Receiver Plate**	Room Temperature	N/A
Human Intestinal Epithelial Cells	Liquid Nitrogen (vapor phase)	N/A

***Precise expiration dates will be specified on the shipped reagents**
****Reagents are for 96-well Transwell kits only**
*****Reagents are cell lot specific**

2.2 HOW SHOULD I STORE THE CRYOPRESERVED PRIMARY CELLS?

Cells should be stored in the vapor phase of a LN_2 tank (< -130°C) until plating.

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3 METHODS QUESTIONS:

3.1 WHAT REAGENTS ARE NEEDED TO RUN THE REPLIGUT® KIT OUTSIDE OF WHAT IS SENT BY ALTIS?

Altis will send the plates with Transwell® inserts, cells, plating reagents, and supportive medias for culture. In addition, a complete protocol for plating is provided. If additional media is needed for any treatments, Altis can provide more by special order. End-users will need to provide pipettors, serological pipettors, standard tissue culture consumables, tissue culture hood, tissue culture incubator (37°C, 5% CO_2), aspiration line, and 37°C water bath.

Altis strongly recommends the use of a multichannel pipettor with appropriately matching sterile channel disposable reservoir for 96-well plating.

3.2 IS SPECIAL EQUIPMENT NEEDED TO MEASURE TEER ON THE TRANSWELLS®?

Yes, an Epithelial Volt/Ohm (TEER) meter is required to measure TEER. We use the EVOM2, EVOM3, and EVOM Auto from World Precision Instruments. Specific probes are needed for individual plate sizes: contact World Precision Instruments for more information. Further information about this equipment can be found here: https://www.wpiinc.com/blog/post/faqs-about-teer-measurement.

We also offer EVOM3 rentals, including the appropriate probe needed for your specific kit. Ask your sales representative for more information.

3.3 WHAT ARE THE SPECIFICATIONS OF THE TRANSWELL® PLATES?

The Transwell® plates used in the RepliGut® Planar platform are listed below.

TW size	TW surface area (cm^2)	Pore size (uM)	Manufacturer	SKU
12-well	1.12	0.4	Corning	3460
24-well	0.33	0.4	Corning	3470
96-well (Gen 0 kits) *Used with 0000 series cells*	0.143	1.0	Corning	3380
96-well (Gen 1 kits) *Used with 1000 series cells*	0.143	0.4	Corning	7369

4 MEDIA QUESTIONS:

4.1 WHAT IS THE GENERAL COMPOSITION OF THE CELL CULTURE MEDIUM?

Media composition is proprietary. If you are looking to avoid a specific component, please contact us.

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4.2 Are there antibiotics or antimycotics in the media?

Yes, there is a combination of antibacterial and antifungal compounds. The antibacterial agents also eliminate mycoplasma. We can provide antibiotic/antimycotic-free RMM by request.

4.3 Is there serum in the media?

Yes, there is fetal bovine serum in both the RGM and RMM. We can provide serum-free RMM by special request.

4.4 How much extra media is shipped with the kits in case of plating error?

Cells should reach confluence in RGM within 6 days followed by maturation phase in RMM lasting another 7-10 days with typical duration of the entire culture period being 16 days. Duodenum cells reach confluence within 8 days followed by an additional 7-10 days with a total of 18 days in culture. Enough media is shipped to culture the cells for the duration of the typical model growth and maturation phases with approximately 20% extra for each.

If additional media is necessary for a study, more can be provided as a special order.

4.5 How often should I change the media?

Media should be changed every 48 hours. See protocol for more information on media changes.

5 General Cell Questions:

5.1 How many cells are provided in each vial?

The number of cells in each vial will vary based on cell lot and can be found on the COA provided with your kit. End users are discouraged from attempting to count cells after thawing prior to plating due to the added delay in plating and fragile nature of cells. All lots are density tested during the quality control process. Cells are provided at correct viable cell density to achieve a successful polarized monolayer with tight junctions within 4-6 days after seeding.

5.2 How do I determine the correct initial seeding density for my cells?

A HISC lot COA will be provided with each kit. Please refer to the COA for recommended seeding densities.

5.3 Is donor information available?

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Yes, donor information is available for all donors. Donor demographics, including sex, race, age, and blood type, will be provided on the cell vial COA.

5.4 What disease status are the cells?

The cells are derived from clinically normal intestine and transplant grade donors. Cells contained in these products are derived from human source material, which users should treat as potentially infectious. Each donor is tested and found non-reactive by an FDA-approved method for the presence of both HIV-1 and HIV-2, hepatitis B virus and hepatitis C virus prior to tissue collection. Testing cannot offer complete assurance that HIV-1, HIV-2, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 6th ed. If you require further information, please contact your site safety officer.

5.5 What passage cells will I receive?

Cells are frozen at passage 9 for colon epithelial cells and passage 4 or 5 for small intestinal epithelial cells.

5.6 How do you ensure there is no contamination?

All lots of cells and media are tested for the presence of bacteria and mold using standardized 7-day cultures in YT or YM media. Mycoplasma is tested using Negative test results are a condition of lot release. Mycoplasma contamination is detected using a Polymerase Chain Reaction and compared with positive and negative controls. Media formulations include antibiotics and antimycotics to further prevent unwanted bacterial or fungal growth.

6 Transwell® Questions:

6.1 What is the volume in the apical and basal compartments of the Transwells®?

Media volumes for the 12-well Transwells® are 1 mL in the apical compartment and 2 mL in the basal compartment. Media can be added to both compartments in a 12-well plate by adding 3 mL media to the apical compartment and allowing it to overflow into the basal compartment. We do not recommend this technique for 96-well plates. Current media volumes for the 96-well Transwells® are 100 µL in the apical compartment and 200 µL in the basal compartment which should be added to each compartment independently.

6.2 What is the best way to monitor the growth of the monolayer cultures?

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Transwell® membranes are made from polyester (PET) and are optically clear. Cells can be visualized using bright field microscopy. Membrane pores are highly visible with and without cells. Altis encourages microscopic visualization of the Transwell® membranes prior to plating the cells to ensure ability to distinguish between a pore and a cell. Altis also encourages observation of the cells every 24 hours post-plating to monitor consistency between the wells and identify any abnormal patterns in cell growth that could prevent experimental executions.

6.3 What is the composition of the scaffold on the Transwell® inserts?

The composition of the scaffold is proprietary. It is extracellular matrix-based and the Transwell® inserts come precoated with a thin layer of the scaffold ready for cell plating. If you are looking to avoid a specific component, please contact us.

7 Cell Growth Phase:

7.1 How long will it take for the cells to become confluent?

The time to reach confluence is dependent on the donor, region, and cell lot. Please see the COA for the expected time to reach confluence. If cells take longer than the time specified on the COA to become confluent, please contact Altis Scientific Support: scientificsupport@altisbiosystems.com.

8 Cell Maturation Phase:

8.1 What is the quantity of each individual differentiated cell type?

Characterization of the model included staining with Alkaline Phosphatase (ALP) for absorptive enterocytes, Mucin2 (MUC2) for goblet cells, and Chromogranin A (CHGA) for enteroendocrine cells. We currently do not perform quantitative evaluation of these markers for every cell lot unless manufacturing method changes.

8.2 How many total cells are expected after culture fully matures?

We have calculated the yield from a subset of cells and approximated the following cell numbers after differentiation:

		# of cells/Transwell	
TW size	**TW surface area (cm^2)**	**Jejunum**	**Transverse Colon**
12-well	1.120	195,000	475,000
96-well	0.143	25,000	60,000

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8.3 How much RNA can be expected to be extracted from differentiated monolayers?

Monolayers can be lysed directly on the Transwell® with RNA Lysis buffer. We recommend using Buffer RLT (Qiagen, Cat#79216) and the associated Qiagen RNA kits. Lysis buffers containing Trizol should not be used, as this can cause rapid degradation of the Transwells® membrane and compromise your samples.

		RNA yield (ng)		
TW size	**TW surface area (cm^2)**	**Average**	**Minimum**	**Maximum**
12-well	1.12	6,000	2,700	9,600
96-well	0.143	300	130	600

8.4 How much protein can be expected to be extracted from differentiated monolayers?

Although RIPA buffer supplemented with protease and phosphatase buffers are ok to use, Altis has not quantified total protein.

8.5 Will mucus accumulate on the Transwell®?

The RepliGut® Planar model is a submerged cell culture model with media changed every 48 hours. Thus, a thick mucus layer will not form; however, goblet cells that release mucus (MUC2 protein) are present in differentiated monolayers and solubilized mucus is present in the apical compartment of the Transwell®.

9 Cell Treatment:

9.1 When should we start our treatment?

Treatment time is project-dependent based on your experimental hypotheses and the cell type you would like to assay. For investigating compound effects on differentiated cells, we suggest beginning treatment at the start of the plateau of TEER, which can be determined in the COA. This is usually after 2-3 days in RMM for colonic cells and ~4 days for small intestinal cells. After the plateau phase of TEER begins in RMM, cells will begin dying naturally 3-4 days later. Therefore, Altis recommends designing your experiment to conclude within this timeframe to reduce the risk of uninterpretable results. If you need help with deciding, please reach out to your Sales Representative or Scientific Support for a free consultation on your experimental design.

9.2 What is the maximal incubation time in buffer like HBSS?

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We suggest culturing RepliGut® cells in the media provided by Altis. However, if a different media is required for your assay, it is recommended that you pilot that treatment. Preliminary studies using HBSS-based buffers show that the cells maintain TEER for a few hours; however, this has not been thoroughly explored by Altis.

10 TEER QUESTIONS:

10.1 WHAT IS TEER?

Transepithelial electrical resistance (TEER) is the measurement of electrical resistance across a cellular monolayer. It is thought to be a very sensitive and reliable method to confirm the integrity and permeability of the tight junctions and of the cell monolayer (See following publication for more information: https://journals.sagepub.com/doi/10.1177/2211068214561025). We measure TEER using a non-invasive probe and an Epithelial Volt/Ohm meter.

10.2 HOW DO YOU CALCULATE CORRECTED TEER VALUES?

Altis calculates “Corrected TEER” using the following formula:

$$(Raw\ measured\ TEER\ of\ sample\ transwell - Raw\ mesaured\ TEER\ of\ blank\ transwell)\ x\ Surface\ Area$$

Blank measurements may vary when using different probes; therefore, we suggest obtaining blank well values for your study/probe. The surface area for each Transwell® is as follows:

TW size	TW surface area (cm^2)
12-well	1.120
24-well	0.33
96-well	0.143

10.3 SHOULD EVERY WELL HAVE THE SAME TEER VALUE? IS THERE AN EXPECTED AVERAGE TEER?

Different donors and regions will have different absolute TEER values but similar trends over time. See the COA for your lot of cells to determine average TEER values during cell QC. Altis strives to have the TEER within 25% CV of the average of the TEER readings at a particular timepoint.

10.4 WHICH TEER VALUES COULD BE EXPECTED AT WHICH TIME POINTS OF THE EXPERIMENT FOR WHICH CELL TYPE?

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During the initial steps of the cell culture, TEER will be very low when the cells are not yet confluent. Once the cells are confluent and switched to RMM, TEER levels are variable depending on time in differentiation, tissue region, plate size, and plating efficiency. See COA for internal data for the specific lot of cells you received.

11 CONTACT INFORMATION:

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RepliGut® is a registered trademark of Altis Biosystems. Transwell® is a registered trademark of Corning Inc.

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