RepliGut® Planar Kit Frequently Asked Questions

Video protocol available online

Also Available:

- Detailed Plating Protocol
- Safety Data Sheets
- Application protocols

www.altisbiosystems.com/Repligut-kit-info/

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scientificsupport@altisbiosystems.com

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1 Kit Component Storage Questions:

1.1 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.

SKU	Reagent	Storage Condition	Expiration*
MED-RGM	RepliGut® Growth Medium (RGM)	4°C	1 month
MED-RMM	RepliGut® Maturation Medium (RMM)	4°C	1 month
TW-RGP-12 TW-RGP-24 TW-RGP-96	RepliGut® Pre-Coated Transwell® Plate	4°C	1 month
IN-CDS	Cell Dissociation Solution (CDS)[a]	4°C	1 month
IN-PBS	Sterile 1X Phosphate Buffered Saline (PBS)	RT	Lot Specific
TW-RGP-12-RP TW-RGP-24-RP TW-RGP-96-RP	Receiver Plate	RT	N/A
TW-RGP-96-ST	Culture Plate Sealing Tape[b]	RT	N/A
TW-RGP-96-GP	Reservoir Plate[b]	RT	N/A
HISC-DUO/Dx [c] HISC-JEJ/Dx [c] HISC-ILE/Dx [c] HISC-AC/Dx [c] HISC-TC/Dx [c] HISC-DC/Dx [c]	Region-specific Human Intestinal Epithelial Stem Cells (HISC)	LN_2 (Vapor Phase)	N/A

Expiration dates are located on the labels of the reagents themselves. Please plan experiments accordingly.

[a] Included in kits with 0000 Series HISC lots

[b] Included in 96-well plate kits only

[c] Denotes the donor number

1.2 How should I store the cryopreserved primary cells?

Cells should be stored in the vapor phase of an LN2 tank (< -130°C) until plating.

2 Methods Questions:

2.1 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 media for culture. In addition, a QR code is provided to direct you to resources like protocols for plating and SDSs. If additional media is needed for any treatment, Altis can accommodate by special order. End-users will need to provide pipettors, sterile filtered tips, serological pipettors, standard tissue culture consumables, centrifuge, 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 an appropriately matching sterile channel disposable reservoir for 96-well plating.

2.2 Is special equipment needed to measure TEER on the Transwells®?

Yes, an Epithelial Volt/Ohm (TEER) meter is required to measure TEER. Altis uses EVOM2, EVOM3, and EVOM Auto from World Precision Instruments. Specific probes are required dependent on plate size: contact World Precision Instruments (WPI) for more information. Further information about this equipment can be found on WPI's website.

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

2.3 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 (µM)	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	3392
96-well (Gen 1 kits) *Used with 1000 series cells*	0.143	0.4	Corning	7369
96-well (Gen 2 kits) *Used with 0000 series cells*	0.143	0.4	Corning	7369

3 Media Questions:

3.1 What is the general composition of the cell culture medium?

Altis Media composition is proprietary. Please contact us at scientificsupport@altisbiosystems.com if media composition knowledge is required for your experimental design.

3.2 What is the osmolarity of the cell culture medium?

The osmolarity of RepliGut® Growth Medium and RepliGut® Maturation Medium typically falls within the range of 320-330 mOsm/kg.

3.3 Are there antibiotics or antimycotics in the media?

A combination of antibacterial and antifungal compounds are present in both RGM and RMM. The antibacterial agents eliminate mycoplasma. Antibiotic/antimycotic-free RMM can be provided upon request.

3.4 Is there serum in the media?

Fetal bovine serum is present in RGM, RMM, and Altis Freezing Media. RGM contains 7% FBS, RMM contains 8% FBS, and Altis Freezing Media contains 44% FBS. Serum-free RMM can be provided upon request.

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

Cells should reach confluence in RGM between 4-10 days post-plating, depending on region. Following confluence, cells will be in RMM an additional 7-10 days with typical duration of the entire culture period being 16-18 days. Enough media is shipped to culture the cells for the duration of the growth and maturation phases including a roughly 20% surplus of both media types.

If additional media is necessary for specific study design, more can be provided upon special order.

3.6 How often should I change the media?

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

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4 General Cell Questions:

4.1 How many cells are provided in each vial?

The number of cells in each vial varies based on cell lot and can be found in the COA provided with your kit. Counting the cells after thawing is discouraged due to the added delay in plating and fragile nature of the cells. All lots are density tested during the quality control process. Cells are provided at an optimized viable cell density to achieve a polarized monolayer with tight junctions within 4-10 days post-plating.

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

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

4.3 Is donor information available?

Information is available in the provided COA included in your kit covering demographics such as sex, race, age, and blood type. HLA typing is available upon request.

4.4 What disease status are the cells?

The cells are derived from clinically non-diseased intestines from 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.

4.5 What passage cells will I receive?

Refer to the COA provided in your kit for the lot specific passage at which your cells were frozen. If this information is pertinent prior to culturing based on your experimental design, please reach out to us at scientificsupport@altibiosystems.com.

4.6 How do you ensure there is no contamination?

Media formulations include antibiotics and antimycotics to prevent bacterial and fungal growth. All cell lots are tested for the presence of bacteria and mold using standardized 7-day cultures in YT or Malt Extract media. Mycoplasma contamination is evaluated using a PCR method and compared with positive and negative controls.

5 Transwell® Questions:

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

Plate Format	Apical (Transwell) Volume (per well)	Basal (Receiver) Volume (per well)	Total Media Volume (per plate)[a]
12-well	1 mL	2 mL	40 mL
24-well	250 µL	750 µL	28 mL

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96-well	100 µL	200 µL	34 mL, 17mL[b]

[a] Includes some excess volume for pipetting
[b] Volume for 96 well trial kits

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

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 abnormal cell growth patterns that could prevent experimental executions.

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

It is an extracellular matrix-based scaffold, but exact composition of the scaffold is proprietary. The Transwell® inserts come precoated with a thin layer of the scaffold by Altis. If you are looking to avoid a specific component, please contact us at scientificsupport@altisbiosystems.com.

6 Cell Growth Phase Questions:

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

The time to reach confluence is between 4-10 days, dependent on the donor, region, and cell lot. Please see the COA provided in your kit for the expected time to reach confluence. If cells take longer than the time specified in the COA to become confluent, please contact us at scientificsupport@altisbiosystems.com.

7 Cell Maturation Phase Questions:

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

Characterization of the model includes staining with Alkaline Phosphatase (ALP) for absorptive enterocytes, Mucin2 (MUC2) for goblet cells, and Chromogranin A (CHGA) for enteroendocrine cells. Altis does not perform quantitative evaluation of these markers for every cell lot currently.

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

Altis has 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	1.12	195,000	475,000
96-well	0.143	25,000	60,000

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7.3 How much RNA can be extracted from differentiated monolayers?

Monolayers can be lysed directly in the Transwell® with RNA lysis buffer. We recommend using Buffer RLT (Qiagen, Cat# 79216) and their associated Qiagen RNeasy kits. Buffers containing Trizol should not be used, as this can cause rapid degradation of the Transwell® membranes.

		Total RNA yield (ng/Transwell®)	
TW size	**TW surface area (cm^2)**	**Average**	**Minimum**
12-well	1.12	6,000	2,700
96-well	0.143	300	130

7.4 How much protein can be extracted from differentiated monolayers?

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

7.5 Will mucus accumulate on the Transwell®?

The RepliGut® Planar model is a submerged cell culture model with media changes 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®.

8 Cell Treatment Questions:

8.1 When should we start our treatment?

Treatment time is project-dependent based on your experimental hypotheses and the cell type being assayed. For investigating compound effects on differentiated cells, we suggest beginning treatment at the start of the TEER plateau for colonic cells and when the cultures have formed a functional barrier for small intestinal cells. This is usually after 2-3 days in RMM for colonic cells and ~4 days for small intestinal cells. After the TEER plateau phase begins in RMM, cells will begin dying naturally 3-4 days later. Therefore, Altis recommends designing your treatment time to conclude within this timeframe for optimal results. For any experimental design questions, please reach out to your Sales Representative or email us at scientificsupport@altisbiosystems.com to schedule a free 1-hour consultation with an Altis Scientist as provided with all kit purchases.

8.2 What is the maximum incubation time in buffer like HBSS?

We suggest culturing RepliGut® cells in the media provided by Altis. However, if a different media is required for your assay, it is recommended to conduct a pilot study with that specific media and treatment period. Exposure to HBSS-based buffers have only been tested up to four hours in RepliGut® without negative impacts on barrier.

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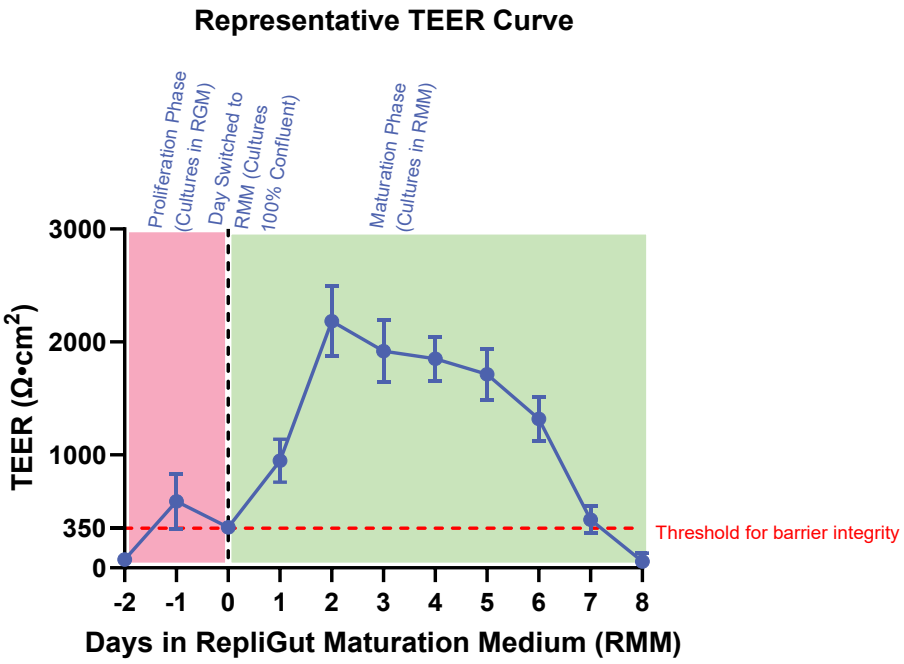
9 Interpreting COA TEER Graphs:

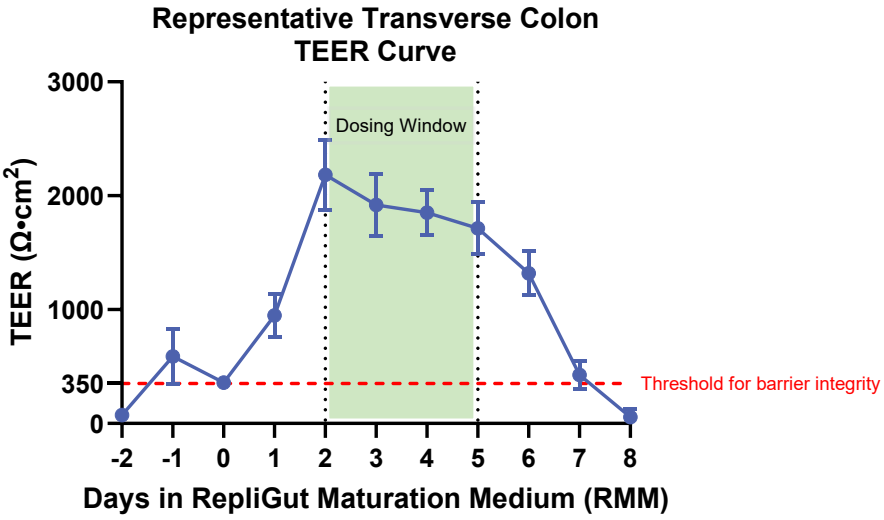
9.1 How do I interpret TEER graphs for cell culturing?



Based on QC data collected to date, optimal results are obtained when cultures are switched to RepliGut® Maturation Medium after at least 4 days in culture. After at least 4 days in culture, confluent cultures are switched to RMM. The cells will polarize and form tight monolayers over the next few days. For small intestine 1000 series HISC lots, it is recommended to wait an additional day (24 hours) after reaching confluence before switching to RMM.

Magnitude of TEER can vary between donors, regions, and lots. Please refer to the CoA for the cells you received for expected TEER values.

9.2 How do I interpret TEER graphs for dosing differentiated colon cells?



Altis recommends beginning treatments at the start of the TEER plateau. For large intestine 0000 series cell lots, Altis typically recommends compound dosing after 2 days in RepliGut® Maturation Medium. For large intestine 1000 series cell lots, it is recommended to dose after 2-3 days in RMM. Reference the COA provided in your kit for the lot-specific plateau window.

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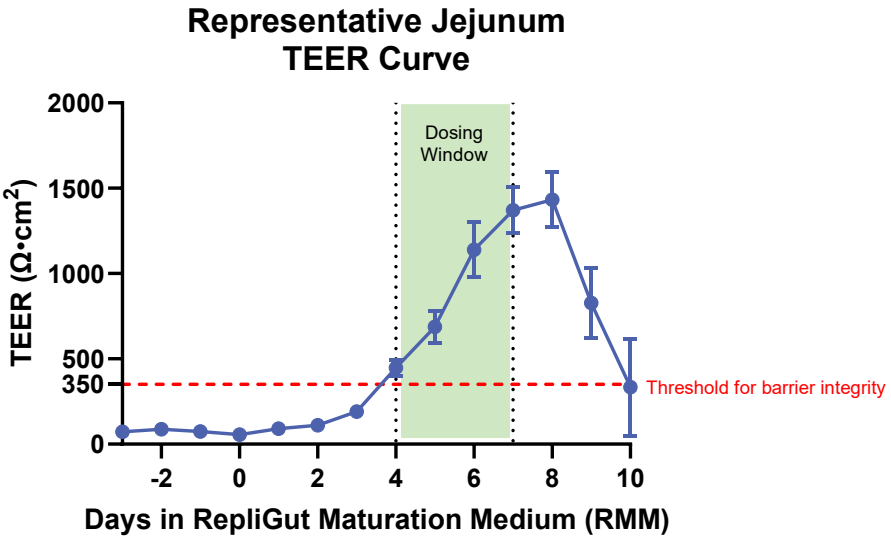
9.3 How do I interpret TEER graphs for dosing differentiated small intestinal cells?



Altis recommends beginning treatments as the cultures are reaching peak TEER. For small intestine cell lots, Altis typically recommends compound dosing after 4 days in RepliGut® Maturation Medium. Reference the COA provided in your kit to determine optimal experimental timeline.

10 TEER Questions:

10.1 What is TEER?

Transepithelial electrical resistance (TEER) is the measurement of electrical resistance across the two sides of the Transwell®. It is thought to be a sensitive and reliable method to evaluate the integrity of the tight junctions in the culture's 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. Blank well values should be measured as a cell-free Transwell® with the appropriate volume of pre-warmed media. The surface area for each Transwell® is as follows:

TW size	TW surface area (cm^2)
12-well	1.12
24-well	0.33
96-well	0.143

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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 provided in your kit for your cell lot to determine average TEER values. Altis strives to have TEER values within 25% CV of the average of the TEER readings at any particular timepoint.

10.4 What TEER values should be expected?

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

11 Contact Information:

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