RepliGut® Planar Kit Plating and Culturing Procedure (12-Well Format)

This protocol applies to the following kits with 0000 series HISC cell lots (i.e. HITC-01-0001)*:

Product #	Product Name
RGP-12W-DUO	RepliGut® Planar - 12 Well - Small Intestine - Duodenum
RGP-12W-JEJ	RepliGut® Planar - 12 Well - Small Intestine - Jejunum
RGP-12W-TC	RepliGut® Planar - 12 Well - Transverse Colon
RGP-12W-AC	RepliGut® Planar - 12 Well - Ascending Colon
RGP-12W-DC	RepliGut® Planar - 12 Well - Descending Colon

*Do not use this protocol for HISC cell lots with a one as the first digit in the last 4 numbers (e.g. HITC-01-1001)

Reagents provided in each kit:

Reagent	Unit	Storage Condition
RepliGut® Growth Medium	200mL	4°C (warm to 37°C before use)
RepliGut® Maturation Medium	200mL	4°C (warm to 37°C before use)
RepliGut® 12-well Pre-Coated Transwell® Plate*	1X 12-Well Plate	4°C
Cell Dissociation Solution	0.5mL	4°C (warm to 37°C before use)
Sterile 1X Phosphate Buffered Saline (PBS)	30mL	Room Temperature
Region Specific Human Intestinal Epithelial Cells	Cell lot dependent	Liquid Nitrogen (vapor phase)

* Please note that the plates in this kit have a 10–12-day shelf life from the time of receipt. The actual date of expiration will be noted on the plate itself. The Human Intestinal Epithelial Cells must be plated within this time.

1. Rinsing RepliGut® 12-well Pre-coated Transwell® Plate

Note: Perform these steps immediately before use:

1. Place the 12-well pre-coated Transwell® plate into a sterile tissue culture hood. Add 1 mL of sterile 1X PBS to apical compartment of each well being used, making sure not to form bubbles between the membrane and PBS solution. If bubbles appear, gently tap the plate, or use a p200 pipettor to remove the bubbles.
2. Aspirate 1X PBS from apical compartment of each well being used.
    a. Note: This is only a rinse, duration that 1X PBS sits on apical surface is irrelevant.
3. Allow Transwell® inserts to dry with the lid off the plate in the tissue culture hood while frozen vials are being processed.

2. Plating Cells from Frozen

This protocol is for plating a full 12-well RepliGut® Planar Transwell® plate. The number of cryovials to plate an entire 12-well Transwell® plate is dependent on the cell lot. Please refer to the COA for seeding recommendations.

2.1. Prior to Cell Plating:

1. Warm Cell Dissociation Solution (for use in Step 10) in 37°C water bath.
2. Warm appropriate volume of RepliGut® Growth Medium in 37°C water bath.
    a. Note: Avoid repeated heating and cooling of RepliGut® Growth Medium and RepliGut® Maturation Medium.

2.2. Cell Plating Procedure:

Note: This plating protocol is for 1 full 12-well RepliGut® Planar Transwell® plate. If multiple plates are being plated at the same time, prepare 1x15mL conical with 3 mL RepliGut® Growth Medium per plate being plated (Step 1 below) and only add cells for one full plate in each conical tube. Cell solutions can be combined after the cell resuspension has been thoroughly mixed (after Step 11).

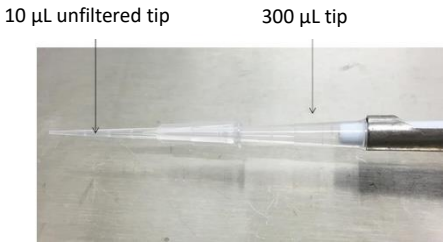
1. Add 3 mL of warm RepliGut® Growth Medium to a 15 mL conical tube.
2. Remove cryovials of intestinal epithelial cells from liquid nitrogen storage.
3. Place cryovials in 37°C water bath until thawed (about 2 minutes).
    a. Note: DO NOT keep in water bath for more than 3 minutes.
    b. Note: Remove from the water bath as soon as the last sliver of ice thaws.
4. Transfer the cells from the cryovials to the 15 mL conical tube containing 3 mL warm RepliGut® Growth Medium with a 1 mL pipettor.
    a. Rinse each cryovial with 1 mL of fresh RepliGut® Growth Medium and transfer to the 15 mL conical tube.
5. Centrifuge conical tube at 600×g for 1 minute at room temperature.
6. Carefully aspirate supernatant, making sure not to disturb the cell pellet.
7. Using a serological pipettor, quickly add 5 mL of 1X PBS to the conical tube to disrupt the cell pellet. Make sure the pipet tip does not contact the cell solution.

   a. Note: The cell pellet does not need to be disassociated, but just dislodged from the bottom of the tube for this rinse step.
8. Centrifuge conical tube at 600×g for 1 minute at room temperature.
9. Carefully aspirate supernatant, making sure not to disturb the cell pellet.
10. Resuspend the cell pellet in warm Cell Dissociation Solution using Table 1 below for required volume, and pipet solution up and down 10 times to thoroughly mix. Place tube upright in 37°C water bath for 3 minutes.

Table 1.

# of Cell Vials per 15 mL Conical	Volume of Cell Dissociation Solution
1	150 µL
2	150 µL
3	225 µL
4	300 µL

11. After 3 minutes, remove tube from the water bath and pipet cells up and down at least 25 times with a 10 µL unfiltered pipet tip over a 300 µL pipet tip (see image below). Note: Allow the pipet tip to fully fill and empty between pipetting. Failure to do so might result in insufficient dissociation.



12. Add 12.2 mL of warm RepliGut® Growth Medium to the conical tube and rinse the double pipet tip used in Step 11 by pipetting up and down a few times to recover any cells adhering to the tip.
13. Fill the basal compartment of 12 Transwells® with 2 mL of warm RepliGut® Growth Medium.
14. Mix the cell solution from Step 12 15-20 times in the 15 mL conical tube with a 1 mL pipettor, moving the pipet tip up and down while pipetting. Plate 1 mL of cell suspension from the conical tube into the apical compartment of each membrane support with a 1 mL pipettor.
    a. Note: Be sure to maintain a homogeneous cell solution by pipetting up and down 5 times between plating each well. It is critical that the cell solution is thoroughly mixed to achieve consistent plating.
    b. Note: Avoid introducing bubbles when mixing and plating the cell suspension.
15. Slowly and carefully move seeded Transwell® plate to 37°C tissue culture incubator.

3. CULTURE AND MATURATION

1. Observe confluence of cultures every 24 hours. Transepithelial Electrical Resistance (TEER) can be measured as early as 24 hours post-plating.
    a. Note: Day of plating is considered Day 0.
2. Change media on apical and basal sides of the membrane supports 48 hours post-plating, and then every 48 hours with RepliGut® Growth Medium until cells are 100% confluent.
    a. Carefully aspirate media from the basal, then apical side of each of the Transwells®. Be careful not to puncture the membrane or aspirate cells when aspirating from apical compartment.
    b. Gently add 3 mL of warmed media to the apical compartment, letting media overflow into the basal compartment.
    c. Note: Add media to the side/wall of the membrane support, not directly onto the middle of the membrane, as to not damage the monolayers.

3. When monolayers become 100% confluent, after measuring TEER, change media to RepliGut® Maturation Medium on apical and basal sides of the Transwells® as in Step 2.
4. Change media in apical and basal compartments with RepliGut® Maturation Medium every 48 hours.

LIMITED GUARANTEE: Altis guarantees performance only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to these recommendations including the use of alternative media, growth factors, reagents or protocols, will void performance guarantees. RepliGut® is a registered trademark of Altis Biosystems. Transwell® is a registered trademark of Corning Inc.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro diagnostic procedures. **WARNING:** Cells contained in these products are derived from human source material, 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 the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed. If you require further information, please contact your site safety officer.

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