RepliGut® Planar Kit Plating and Culturing Procedure (96-Well Format)

This protocol applies to the following kits with 1000 series HISC cell lots (i.e. HITC-01-1000)*:

Product #	Product Name
RGP-96W-DUO	RepliGut® Planar - 96 Well - Small Intestine - Duodenum
RGP-96W-JEJ	RepliGut® Planar - 96 Well - Small Intestine - Jejunum
RGP-96W-TC	RepliGut® Planar - 96 Well - Transverse Colon
RGP-96W-AC	RepliGut® Planar - 96 Well - Ascending Colon
RGP-96W-DC	RepliGut® Planar - 96 Well - Descending Colon
RGP-96W-JEJ-Trial	RepliGut® Planar - 96 Well - Small Intestine – Jejunum – ½ plate
RGP-96W-TC-Trial	RepliGut® Planar - 96 Well - Transverse Colon – ½ plate

*Do not use this protocol for HISC cell lots with a zero as the first digit in the last 4 numbers (e.g. HITC-01-0001)

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® 96-well Pre-Coated Transwell® Plate*	1X 96-Well Plate	4°C
Sterile 1X Phosphate Buffered Saline (PBS)	30mL	Room Temperature
Culture Plate Sealing Tape	4 strips	Room Temperature
Receiver Plate	96-well plate	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® 96-well Pre-coated Transwell® Plate

Note: Perform these steps immediately before use:

1. Place the 96-well pre-coated Transwell® plate into a sterile tissue culture hood. Add 100 µL of sterile 1X PBS to apical compartment of all wells using a multichannel pipettor, 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 96-well RepliGut® Planar Transwell® plate. The number of cryovials to plate an entire 96-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 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 96-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).

Note: For RGP-96W-JEJ-Trial and RGP-96W-TC-Trial kits which contain enough cells for half of a plate, make the following protocol changes to the Cell Plating Procedure:

1. Resuspend the cell pellet into 1 mL in Step 10.
2. Add 4.5 mL RepliGut® Growth Medium to the cell suspension in Step 12.
3. Add 10 mL RepliGut® Growth Medium to reservoir in Step 13 and pipette 200 µL into the basal compartment of 48 Transwells® using a multichannel pipettor.
4. Fill the unused 48 Transwells® with 1X PBS to minimize evaporation.

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 2 minutes 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 2 minutes at room temperature.
9. Carefully aspirate supernatant, making sure not to disturb the cell pellet.
10. Resuspend the cell pellet in 2 mL of warm RepliGut® Growth Medium using a 1 mL pipettor.
11. Mix the cell suspension 25 times with a 1 mL pipettor. Then mix 25 times with the P1000 pipette tip touching the bottom of the conical to create shear force when pipetting up and down.
12. Add 9 mL of warm RepliGut® Growth Medium to the cell suspension from Step 11.
13. Add 20 mL of warm RepliGut® Growth Medium to a media reservoir, and then pipet 200 µL into the basal compartment of each Transwell® using a multichannel pipettor.
14. Mix the cell solution from Step 12 15-20 times in the 15 mL conical tube with a 1 mL pipettor. Then using the 1 mL pipettor, transfer the cell solution 1 mL at a time to a sterile, empty media reservoir, pipetting the cell suspension in the conical tube up and down in between each transfer.
   a. Note: Be sure to take all the cell suspension from the conical tube. Transfer any remaining cell solution from the conical tube using a p200 pipettor.
   b. Note: Avoid introducing bubbles when mixing and transferring the cell suspension.
15. Mix the cell solution in the reservoir 15-20 times with a multichannel pipettor, moving the pipet tips up and down while pipetting.
   a. Note: It is critical that the cell solution is thoroughly mixed to achieve consistent plating.
16. Plate 100 µL of homogenous cell suspension from the media reservoir into the apical compartment of each membrane support with a multichannel pipettor.
   a. Note: Make sure to mix the cell suspension in the media reservoir well during plating to ensure homogenous cell suspension, by pipetting up and down 5 times between plating each column with a multichannel pipettor.
   b. Note: Make sure there are no bubbles present in the apical compartment between the membrane and the cell solution. If bubbles are present, gently tap the plate to remove them.
   c. Note: A single channel p200 µL pipettor may be used but may increase variability of quantity of cells seeded per Transwell®.
17. Wrap plate edges with Culture Plate Sealing Tape, sealing plate lid securely to base plate to limit media evaporation.
18. 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.
   b. Note: Measurement of TEER on 96-well Transwells® requires the use of a specialized TEER probe for Corning HTS 96-well Transwells (contact World Precision Instruments for more information).
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 remove the culture plate sealing tape and keep for re-use throughout the culture period. If a tape strip becomes unusable, replace it with a fresh strip (replacements included with kit).
   b. Move the Transwell® insert into the receiver plate and carefully aspirate media from the 96-well base plate (basal compartment).

c. Carefully aspirate media from the apical side of the membrane supports. Be careful not to puncture the membrane or aspirate cells when aspirating from apical compartment.
d. Place the Transwell® insert back into the 96-well base plate.
e. Gently add 100 µL of warmed media to the apical compartment, then add 200 µL of media to the basal compartment. Add media to the side/wall of the membrane support, not directly onto the middle of the membrane, as to not damage the monolayers
f. Reseal the plate by wrapping plate edges with culture plate sealing tape, sealing plate lid securely to base plate to limit media evaporation.

3. When monolayers become 100% confluent, after measuring TEER, change media to RepliGut® Maturation Medium on apical and basal sides of the Transwells® using Steps 2a-2f above.
    a. Note: For small intestine kits, wait an additional day (24 hours) after reaching confluence before switching to RepliGut® Maturation Medium.
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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