RepliGut® Planar Kit Technical Guide and Protocol

Video protocol available online

Also Available: Frequently Asked Questions | Safety Data Sheets | Application protocols

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

REPLIGUT® PLANAR KIT INFORMATION

Table 1: The information applies to the following RepliGut® Planar kits

SKU	Product Name	Transwell® Manufacturer
RGP-12W-[xxx][a]	RepliGut® Planar - 12 Well - [Region][b]	Corning, 3460
RGP-24W-[xxx][a]	RepliGut® Planar - 24 Well - [Region][b]	Corning, 3470
RGP-96W-[xxx][a]	RepliGut® Planar - 96 Well - [Region][b]	Corning, 3392 Corning, 7369
RGP-96W-[xxx][a] -Trial	RepliGut® Planar - 96 Well Trial Kit - [Region][b]	Corning, 3392 Corning, 7369

[a]"DUO" for duodenum, "JEJ" for jejunum, "ILE" for ileum, "AC" for ascending colon, "TC" for transverse colon, or "DC" for descending colon
[b] Duodenum, Jejunum, Ileum, Ascending Colon, Transverse Colon, Descending Colon

Table 2: Individual kit components

SKU	Reagent	Units	Storage Condition
MED-RGM	RepliGut® Growth Medium (RGM)	200 mL, 100 mL[a]	4°C
MED-RMM	RepliGut® Maturation Medium (RMM)	200 mL, 100 mL[a]	4°C
TW-RGP-12 TW-RGP-24 TW-RGP-96	RepliGut® Pre-Coated Transwell® Plate	1 plate	4°C
IN-CDS	Cell Dissociation Solution (CDS)[b]	0.5 mL	4°C
IN-PBS	Sterile 1X Phosphate Buffered Saline (PBS)	30 mL	RT
TW-RGP-12-RP TW-RGP-24-RP TW-RGP-96-RP	Receiver Plate	12-well plate 24-well plate 96-well plate	RT
TW-RGP-96-ST	Culture Plate Sealing Tape[c]	4 strips	RT
TW-RGP-96-GP	Reservoir Plate[c]	96-well plate	RT
HISC-DUO/Dx[d] HISC-JEJ/Dx[d] HISC-ILE/Dx[d] HISC-AC/Dx[d] HISC-TC/Dx [d] HISC-DC/Dx[d]	Region-specific Human Intestinal Epithelial Stem Cells (HISC)	Vials cell lot dependent	LN_2 (Vapor Phase)

Expiration dates are located on the labels of the reagents themselves. Please plan experiments accordingly.
[a] Volume for 96-well trial kits
[b] Included in kits with 0000 Series HISC lots
[c] Included in 96-well plate kits only
[d] Denotes the donor number

Table 3: Human Intestinal Stem Cell (HISC) Lot Series Identification: The first number in the end 4-digit number sequence indicates the HISC lot series described throughout this protocol.

Series	Example
0000 Series	HITC-01-**0**001
1000 Series	HITC-01-**1**001

PLATING KIT VOLUMES

Table 4. Reference Volumes

Plate Format	Apical (Transwell®) Volume (per well)	Basal (Receiver) Volume (per well)	Total RGM Volume (per plate)[a]
12-well	1 mL	2 mL	40 mL
24-well	250 µL	750 µL	28 mL
96-well	100 µL	200 µL	34 mL, 17mL[b]

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

Table 5. Cell Dissociation Solution (CDS) Volume (for 0000 HISC Lot series only)

# of Cell Vials (per plate)[a]	CDS Volume
1	150 µL
2	150 µL
3	225 µL
4	300 µL

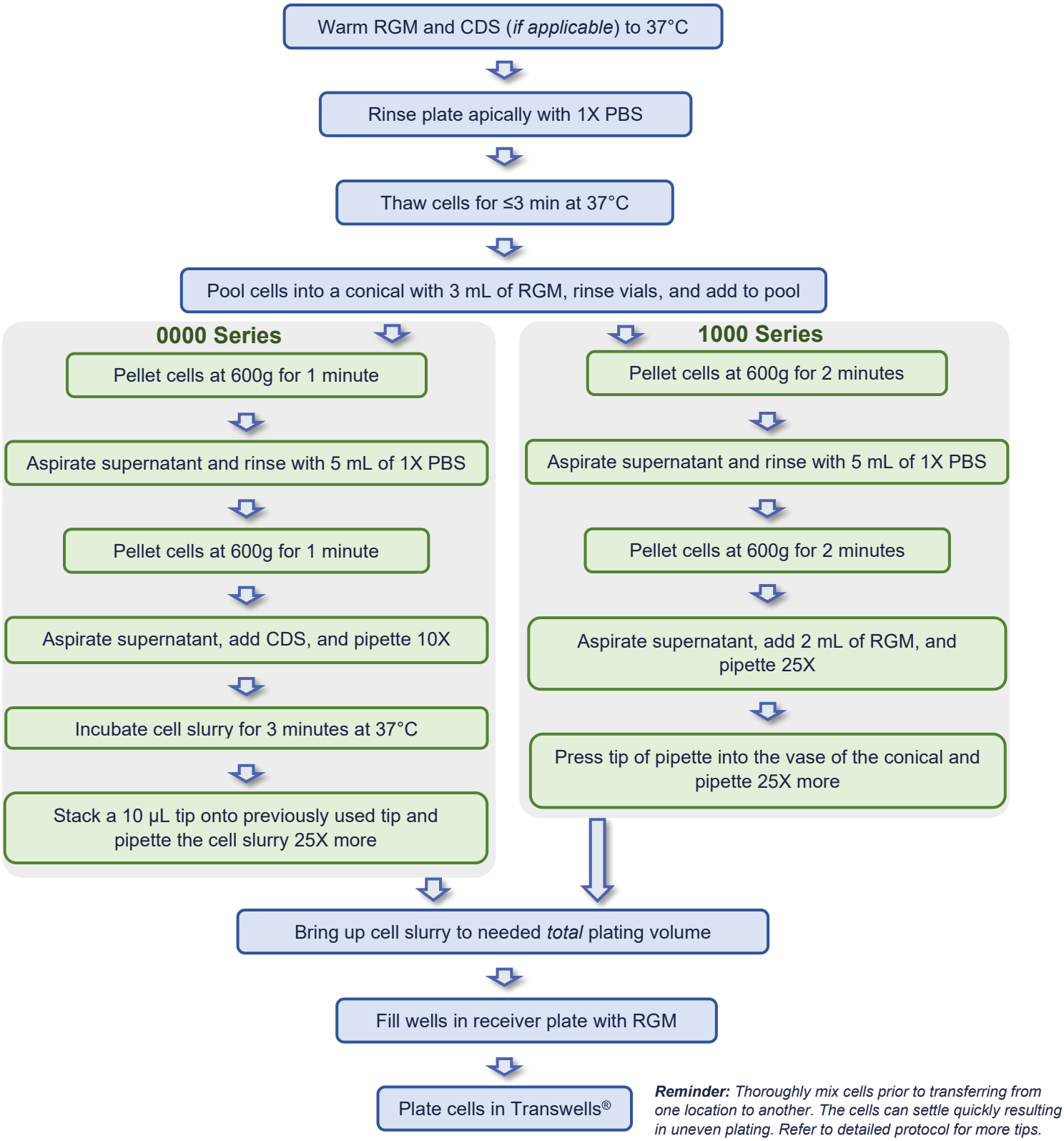
[a] Consult the HISC lot's CoA to determine the number of cell vials required per plate.

Table 6. Total Cell Suspension Volumes for Plate Formats

HISC Lot Series	Plate Format	Total Cell Suspension Volume (per plate)
0000 Series	12-well	12.2 mL
	24-well	6.2 mL
	96-well	10.2 mL, 5.1 mL[a]
1000 Series	12-well	13 mL
	24-well	6.5 mL
	96-well	11 mL, 5.5 mL[a]

[a] Volume for 96-well trial kits

QUICK PLATING PROTOCOL

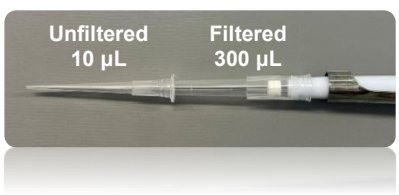


Reminder: *Thoroughly mix cells prior to transferring from one location to another. The cells can settle quickly resulting in uneven plating. Refer to detailed protocol for more tips.*

DETAILED PLATING PROTOCOL

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

Procedure	Tips
1. Rinse Transwells® with Sterile 1X Phosphate Buffered Saline (PBS) warmed to RT. Refer to **Table 4** for apical volumes.	If bubbles occur between the surface of the well and 1X PBS, tap the plate until bubbles release.
2. Aspirate PBS and allow the Transwells® to dry with the lid completely off.	Duration of rinse is irrelevant. Plate can dry while cells are being prepared.
3. Warm an appropriate volume of RepliGut® Growth Medium (RGM) and Cell Dissociation Solution (CDS, *0000 Series* only) in a 37°C water bath. Refer to **Table 4** and **5** for total volumes needed.	Refer to **Table 3** for identifying your HISC Lot series.
4. Aliquot 3 mL of pre-warmed RGM into a 15 mL conical.	Each 15mL conical with 3 mLs of media supports plating a single plate. Use another conical for each additional plate. Cells can be combined just prior to plating to achieve plating uniformity.
5. Place cryovials in 37°C water bath until thawed.	Cells should be thawed for **≤3 minutes**, until a sliver of ice is left. *Longer thawing times reduce cell viability.*
6. Transfer cells from vials using filtered 1000 µL pipet tip to the 15 mL conical containing 3 mL of prewarmed RGM and rinse vials with 1 mL of RGM.	Add volume used to rinse vials to the 3 mL solution of RGM to maximize cell yield.
7. Prepare cells for plating using the following method based on lot series:	
0000 Series	
i. Centrifuge cells at 600g for 1 minute and aspirate the supernatant.	
ii. Rinse pellet with 5 mL 1X PBS. Do not dissociate the pellet.	Avoid touching the pipette tip to the rinse solution.
iii. Centrifuge cells at 600g for 1 minute and aspirate the supernatant.	
iv. Add CDS to the pellet and pipette 10X with a filtered 300 µL tip. Refer to **Table 5** for appropriate volume. *Leave the tip on the pipet and keep sterile for Step vi.*	Keep your pipette volume the same for remainder of Step 7 and allow the pipet tip to fully fill and purge between pipetting.
v. Incubate cells in a 37°C water bath for 3 minutes.	
vi. Add a 10 µL unfiltered tip to the used filtered 300 µL tip from Step 7 iv.	
vii. With the tip flush with the bottom of the tube, dissociate the cells by pipetting 25X through the 10 and 300 µL tips.	Allow the tips to fully fill and purge between pipetting. Suspension should be “milky” in appearance with no observable clumps. Save tips and rinse in media in Step 8.



For protocol questions or assistance, contact Altis Scientific Support:
scientificsupport@altisbiosystems.com

1000 Series

i. Centrifuge cells at 600g for 2 minutes and aspirate the supernatant.

ii. Rinse pellet with 5 mL 1X PBS. Do not dissociate the pellet.

Avoid touching the pipette tip to the rinse solution.

iii. Centrifuge cells at 600g for 2 minutes and aspirate the supernatant.

iv. Add 2 mL of RGM to the pellet and pipette 25X using a filtered 1000 µL pipet tip.

Set your pipette volume to 1000 µL for all of Step 7 and allow the pipet tip to fully fill and purge between pipetting. Save tips and rinse in media in Step 8.

v. Place the pipette tip flush to the base of the conical and pipette 25X.

8. Bring the volume of cell suspension in Step 7 up to the appropriate volume needed to plate cells in the Transwells® with prewarmed RGM. Refer to **Table 6** for total cell suspension volumes.

9. Add the appropriate volume of prewarmed RGM to fill each well in the receiver plate. Refer to **Table 4** for basal compartment volumes.

For Trial kits, fill the unused wells with equal volumes of PBS to reduce evaporation.

10. Pipette cell solution 20X using a 1000 µL pipet tip and:

Move the tip up and down throughout the suspension when pipetting to assist with mixing – avoid introducing air bubbles.

RepliGut® Planar – 12 & 24 Well

i. Pipette the appropriate volume of cell solution directly into the Transwells® using a 1000 µL pipet tip. Refer to **Table 4** for apical volumes. Pipet the suspension 5-10x between each well.

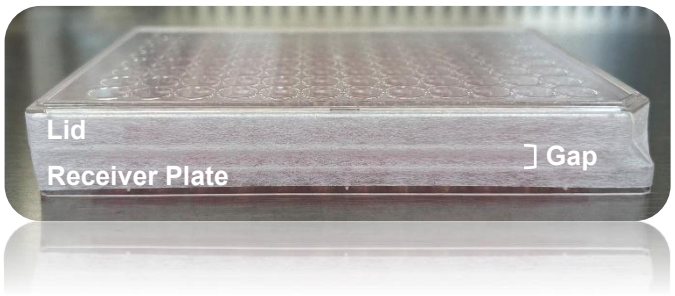
RepliGut® Planar - 96 Well

i. Transfer cell solution 1 mL at a time into an 8-channel reservoir.

ii. Using an 8-channel pipette, transfer 100 µL of cell solution directly into each Transwell® using filtered 300 µL pipet tips. Pipet the suspension 5-10x between each column.

11. Seal plates with Culture Plate Sealing Tape (*if applicable*) and carefully transfer cultures into an incubator at 37°C with 5% CO_2. There will be a gap between the lid and receiver plate when the Transwells® are present as pictured below. Ensure tape covers this gap to reduce evaporation.

The sealing tape is long enough to be applied all the way around the plate and overlap. Curl one end of the tape to create a pull tab for easy removal during media changes. Tape may be reused if it does not become compromised or contaminated.



CULTURE MAINTENANCE AND MATURATION

Measurement of TEER on Transwells® requires the use of a specialized TEER probe for Corning Transwells® found in **Table 1** (contact World Precision Instruments for information on the correct probe). Day of plating is considered Day 0.

Procedure	Tips
1. Observe confluence of cultures every 24 hours using a brightfield microscope and/or Transepithelial Electrical Resistance (TEER).	TEER can be measured as early as 24 hours post-plating. However, accurate readings may not be present until after 1st media change.
2. Change media on apical and basal sides of the Transwells® 48 hours post-plating, followed by every 48 hours with RepliGut® Growth Medium (RGM) until cells are 100% confluent. i. Warm the appropriate volume of RGM. ii. Aspirate media from the apical and basal compartments of the Transwells®. iii. Replenish media to both compartments carefully by ejecting slowly onto the side walls of the wells.	Extending 48 hours or more between media changes can lead to poor cellular growth. For *12-well plates* – media can be added to the apical side, allowing media to pour over into the basal compartment rather than filling individual sides. For *96-well plates* – if the integrity of Culture Plate Sealing Tape is compromised, replace with a fresh tape. The reservoir plate may be used as a holder for the Transwells® during media changes. Ensure the inside of the reservoir plate remains sterile if used. Refer to **Table 4** for volumes. Avoid touching the surface of the cell.
3. When monolayers become 100% confluent, after measuring TEER, change media to RepliGut® Maturation Medium (RMM) using the same methods described in Step 2.	Cultures should not be switched to RepliGut® Maturation Medium until they have been in culture for at least 4 days. For *1000 Series* Small Intestine lots (Duodenum, Ileum, Jejunum), wait an additional 24 hours after cells reach 100% confluence to switch to RMM. *If unsure when to switch cultures to RMM, send daily TEER data to the Altis Scientific Support team for assistance.*
4. Change media in apical and basal compartments with RMM every 48 hours.	

Contact Information:

Sales: info@altisbiosystems.com

Scientific Support: scientificsupport@altisbiosystems.com

