RepliGut® Co-Culture (THP-1) Procedure

These instructions enable co-culture using the following kits:

Altis Kit #	Product Name
CCT-96W	RepliGut® Co-Culture - THP-1 - 96 Well
RGP-96W-TC	RepliGut® Planar - 96 Well - Transverse Colon

Reagents provided in RGP-96W-TC

Please see Altis Biosystems Protocol AB-C-010 for list of reagents and procedures for establishing RepliGut® Transverse Colon epithelium. All protocols can be found on our website:

https://www.altisbiosystems.com/repligut-kit-info/

Reagents provided in CCT-96W

Reagent (Catalog #)	Unit	Storage Condition
RepliGut® Co-Culture Medium – THP-1 (MED-CMT-200)	200 mL	4°C (warm to 37°C before use)
96-Well Receiver Plate (TW-RGP-96-RP)	1 x 96-Well Plate	Room Temperature
Culture Plate Sealing Tape (TW-RGP-96-ST)	4 strips	Room Temperature
Sterile 1X PBS (IN-PBS)	30 mL	Room Temperature

*Expiration dates are reagent specific and are located on the labels of the reagents themselves. Please plan experiments accordingly.

Reagents NOT provided in each kit:

Reagent	Altis Recommended Vendor, Catalog #
THP-1 Cells	ATCC, TIB-202
RPMI-1640 Medium	ATCC, 30-2001
GlutaMAX	Gibco, 35050061
Primocin	InvivoGen, ant-pm-05
Fetal Bovine Serum (FBS)	ATCC, 30-2020
2-mercaptoethanol (2-ME)	Gibco, 21985023
PMA (Phorbol 12-myristate 14-acetate)	Sigma Aldrich, 5.00582 (reconstitute according to manufacturer directions)
LPS (Lipopolysaccharide)	Invivogen, tlrl-peklps (reconstitute according to manufacturer directions)
IFNγ (Interferon gamma)	Peprotech, 300-02 (reconstitute according to manufacturer directions)

Additional Medium Preparation Recommendations:

Medium	Reagent
THP-1 Culture Medium	RPMI-1640 Medium
	Fetal Bovine Serum (FBS) (8%)
	GlutaMAX (2 mM)
	Primocin (100 µg/mL)

1. CULTURE TIMELINES: UNDERSTANDING THE SETUP

Figure 1. Timeline Schematic for RepliGut® - Planar Transverse Colon primary human colonic epithelial cells (RepliGut® -TC) and THP-1-dervied macrophages (THP-1m). Transverse colon culture establishment (green timeline): stem and progenitor cells are seeded on the cell culture inserts of a 96-well Transwell plate. Once confluent, RepliGut® -TC cells are differentiated for 48 hours using RepliGut® Maturation Medium (RMM). Macrophage culture establishment (pink timeline): On the same day that RepliGut-TC cultures are switched to RMM, THP-1 cells are seeded on a separate receiver plate in THP-1 Culture Medium containing PMA to induce differentiation to THP-1m cells. The following day, the Medium is replaced with fresh THP-1 Culture Medium (no PMA) for an additional 24 hours. For inflamed co-culture wells, THP-1m are pretreated with LPS and IFN-γ 4 hours prior to initiation of co-culture with RepliGut® TC. Co-culture (blue timeline): Differentiated RepliGut® TC monolayers in the Transwell inserts and THP-1m in the receiver plate are brought together and co-cultured for 48 hours. For inflamed co-culture, LPS and IFNγ are included in the apical and basal co-culture Medium.

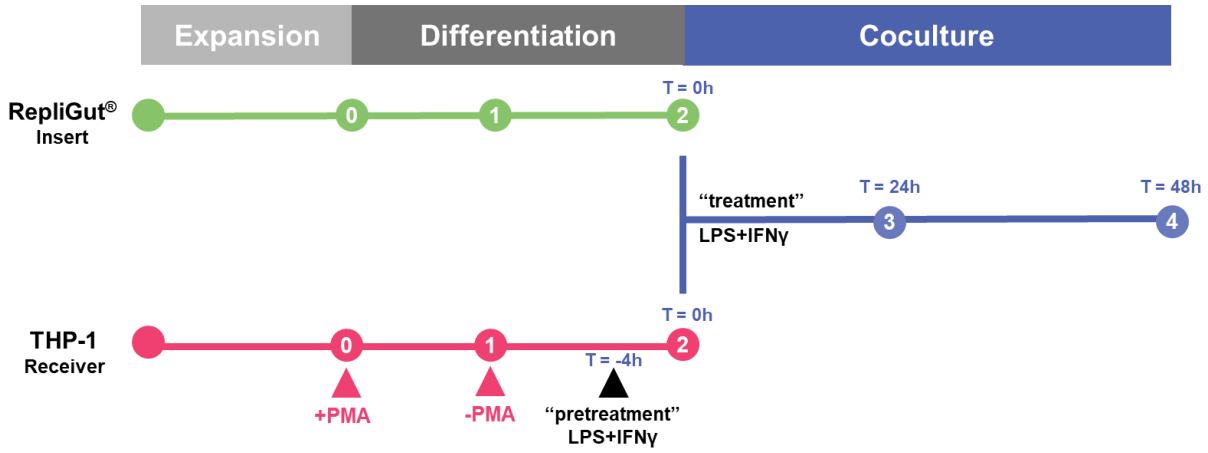

Table 1. Methods Overview – Aligning Cell Culture Timelines

Days	THP-1 Culture	Transverse Colon (TC) Epithelial Cell Culture
Prior Days	Expand THP-1 cells and create cell bank as desired. Thaw cells, complete at least 1 passage post-thaw, and ensure you have enough cells to seed for your experiment. **See "Recommended Approach" in protocol section 3.1*	Seed RepliGut® TC cells in Transwell system provided in the RepliGut® Kit so they come to confluence (Day 0) at the desired time. Consult CoA for time to confluence of specific cell lots.
Day 0 **These THP-1 and TC steps MUST take place on the same day.**	Seed THP-1 cells onto 96 well receiver plate (no cell culture inserts in plate) in medium with PMA to induce macrophage differentiation.	Upon cell confluence, switch culture medium to RMM.
Day 1	Monitor cells for attachment to indicate differentiation of THP-1 cells to THP1m macrophage cells. Perform medium change to remove PMA.	--
Day 2 **T = -4 hours.**	For establishing desired "inflamed" conditions, treat THP-1m cells with LPS and IFNγ.	--
Day 2 **T = 0 hours.**	Combine THP-1m and RepliGut® TC monocultures, according to the methods provided in the protocol below. Treat "inflamed" groups with LPS and IFNγ in CMT.	
Day 3- 4 **T = 24-48 hours.**	Recommended assay window endpoint. TEER and/or desired assays.	

2. Prepare RepliGut® Planar Transverse Colon

The appropriate RepliGut® Planar Kit Plating and Culturing Procedure should be followed for plating and culturing RepliGut® Planar Transverse Colon human intestinal epithelial cells. Please see Altis Biosystems Protocol AB-C-010 for list of reagents and procedures for establishing RepliGut® Transverse Colon epithelium. All protocols can be found on our website:

https://www.altisbiosystems.com/repligut-kit-info/

Recommended: Include some medium-only wells (no cells) while plating epithelial cells to enable THP-1m monoculture controls. Co-culture of RepliGut® TC epithelial cells with THP-1m cells will start after 2 days in RMM (2 days post confluence). Prior to plating RepliGut® TC cells, review Section 1 (Culture Timelines: Understanding the Setup) to ensure proper preparation.

3. THP-1 Cell Preparation

Overview

Purchase THP-1 cells and associated expansion reagents (suggested vendors listed on page 1). Expand the cells in THP-1 Culture Medium supplemented with 2-mercaptoethanol (2-ME) as described below and in accordance with the manufacturer's protocols. To support co-culture studies at passage 11, we recommend cryopreserving THP-1 cells at 2 × 10^6 cells/vial at or before passage 8. To prepare for co-culture, we recommend passaging THP-1 cells at least once post-thaw before seeding them for experiments. A fully confluent T-225 flask of THP-1 cells should yield enough cells for 1–2 96-well co-culture plates.

Approximate timeline for co-culture preparation:

- Day -5: Thaw a single vial of THP-1 cells
- Day -3: Passage THP-1 cells from T-75 flask to T-225 flask
- Day 0: Seed to 96-well receiver plates for co-culture

Users should verify expansion timelines and final cell yields from their own THP-1 cell bank before beginning a co-culture experiment.

Preparing THP-1 for Seeding on Receiver Plates

1. Prepare Culture Medium
    a. Prepare the required volume of THP-1 Culture Medium.
    b. Supplement with 2-ME to a final concentration of 0.05 mM on the day of use.
    c. Warm medium to 37°C before use.
2. Thaw THP-1 Cells
    a. Remove cryovials from liquid nitrogen and thaw in a 37°C water bath for 2–3 minutes, until only a small ice fragment remains.
    b. Transfer contents to a 15 mL conical tube containing 3 mL of THP-1 Culture Medium + 2-ME.
    c. Centrifuge at 600 × g for 2 minutes. Aspirate supernatant.
    d. Rinse pellet with 5 mL of 1× PBS, then centrifuge again at 600 × g for 2 minutes. Aspirate.
    e. Resuspend in 10 mL of fresh THP-1 Culture Medium + 2-ME (per vial thawed).
    f. Transfer 10 mL of cell suspension into a T-75 flask pre-filled with 10 mL of warm THP-1 Culture Medium + 2-ME (total volume = 20 mL).
    g. Incubate at 37°C with 5% CO_2 and monitor cell growth every 1–2 days.
3. Passage THP-1 Cells: THP-1 cells should be passaged when density reaches 8 × 10^5 cells/mL or when visible clumping occurs. (Refer to ATCC visual protocols for guidance: THP-1 - TIB-202.)
    a. Gently pipette to break up cell clumps.
    b. Transfer suspension to a sterile conical tube and centrifuge at 600 × g for 2 minutes.
    c. Aspirate medium and resuspend pellet in fresh, pre-warmed THP-1 Culture Medium + 2-ME.
    d. Transfer cells from the T-75 flask into a new T-225 flask at a 1:3 ratio. Add fresh medium to bring the final volume to 50 mL.
    e. Incubate at 37°C with 5% CO_2 and continue monitoring every 1–2 days.
    - *Note: Avoid seeding at low densities, as THP-1 cells proliferate poorly under such conditions (Bowdish 2011).*

4. THP-1 Cell Seeding and Differentiation in Receiver Plates

Note: THP-1 cells should be plated on the same day that the transverse colon epithelial cells reach confluence and are transitioned to RMM medium. THP-1 culture confluence is not required—only that sufficient cell numbers are available.

1. Prepare Medium:
    a. Prepare appropriate volume of THP-1 Culture Medium. Note: Do NOT supplement with 2-ME from this point onward.
    b. Warm THP-1 Culture Medium to 37°C.
2. Spin Down and Count Cells:
    a. Collect THP-1 cell suspension from flasks into conical tubes
    b. Centrifuge at 600 g RCF for 2 minutes.
    c. Aspirate supernatant and resuspend the cell pellet in a known volume (e.g. 1 mL) of the warmed medium (from Step 1)
    d. Perform a viable cell count.
3. Calculate Required Cell Suspension:
    a. Required plating density: (2.1 x 10^4 cells/well) / (0.1 mL/well) = 2.1 x 10^5 cells/mL.
    b. *Suggestion*: prepare 10% excess cell suspension for plating.
4. Prepare Final Cell Suspension:
    a. Based on the cell count, aliquot appropriate volume of cell suspension to a new sterile conical tube.
    b. Add THP-1 Culture Medium to bring total cell suspension volume up to volume required to plate wells and so that final cell density is 2.1 x 10^5 cells/mL.
    c. Supplement cell suspension with PMA to achieve a final concentration of 100 nM PMA.
        i. Note: Recommended to dilute reconstituted PMA stock to 1000X of final culture concentration prior to adding to medium.
5. Seed Cells:
    a. Gently mix the final cell suspension (from Step 4b) to ensure even distribution.
    b. Dispense 100 µL into each designated well of the 96-well receiver plate.
    c. For epithelial monoculture controls (recommended): add 100 µL of THP-1 Culture Medium + 100 nM PMA (but without cells) to designated control wells.
    d. Seal plate with Culture Plate Sealing Tape and incubate the plate for 24 hours in a 37°C tissue culture incubator.
6. Medium Change:
    a. After 24 hours, observe cells under a brightfield microscope to confirm adherence. Adherence reflects cell differentiation to THP-1 derived macrophages (THP-1m).
    b. Carefully aspirate the PMA-containing medium.
    c. Replace with 100 µL/well of fresh THP-1 Culture Medium (*without 2-ME or PMA*).
7. Rest Cells:
    a. Reseal plate with Culture Plate Sealing Tape
    b. Incubate for an additional 24 hr at 37°C.
    c. Confirm cells remain adhered. Cells are now ready for Pretreatment and/or co-culture.

5. THP-1M Cell Pretreatment

Timing: Perform 20–24 hours after the rest period and 4 hours before co-culture initiation.
Wells designated for “inflamed” conditions require pretreatment with LPS and IFN-γ to induce an inflammatory phenotype.

1. Prepare Medium and Reagents
    a. Warm THP-1 Culture Medium (without 2-ME or PMA) to 37°C.
    b. Prepare pretreatment medium as follows:

i. For “Healthy” conditions:
    1. Use THP-1 Culture Medium alone.
    2. Add vehicle controls as appropriate (e.g., buffer used to reconstitute LPS/IFNγ), if used in “inflamed” condition.
ii. For “Inflamed” conditions:
    1. Supplement THP-1 Culture Medium with:
        a. 10 ng/mL Lipopolysaccharide (LPS)
        b. 25 ng/mL Interferon gamma (IFN-γ)

2. Prepare 1000× LPS and IFNγ Stock Solutions
    a. LPS Stock (1000×):
        i. Desired final concentration: 10 ng/mL
        ii. 1000× stock concentration: 10 µg/mL
        iii. Dissolve LPS powder in water, per manufacturer’s instructions.
        iv. Aliquot and store at -80°C; avoid repeated freeze-thaw cycles.
    b. IFNγ Stock (1000×):
        i. Desired final concentration: 25 ng/mL
        ii. 1000× stock concentration: 25 µg/mL
        iii. Reconstitute using manufacturer-recommended buffer (PBS with 0.5% BSA).
        iv. Aliquot and store at -80°C for long-term use; avoid repeated freeze-thaw cycles.

    Tip: Add LPS and IFNγ to warmed culture medium just before use to avoid protein degradation.
3. Pretreat THP-1 Macrophages
    a. Carefully aspirate medium from each well of the THP-1m-seeded 96-well receiver plate.
    b. Add 100 µL/well of the appropriate medium:
        i. “Healthy” condition: THP-1 Culture Medium with vehicle control
        ii. “Inflamed” condition: THP-1 Culture Medium + 10 ng/mL LPS + 25 ng/mL IFNγ
        iii. Include any additional co-treatments at this time, if applicable.
    c. Seal the plate with Culture Plate Sealing Tape.
    d. Incubate at 37°C, 5% CO_2 for 4 hours.

6. Epithelial-THP-1 Co-Culture

Co-Culture Initiation Protocol (Post-THP-1m Pretreatment)

Timing: Initiate co-culture immediately after 4-hour pretreatment of THP-1m cells with LPS and IFNγ.

1. Warm and Prepare Co-Culture Medium
    a. Warm an appropriate volume of RepliGut® Co-Culture Medium – THP-1 (CMT) in a 37°C water bath.
    b. Prepare the following medium conditions:
        i. Vehicle controls: Add appropriate vehicle (e.g., LPS/IFNγ diluent) to warm CMT.
        ii. Dosed groups: Supplement CMT with:
            1. 10 ng/mL LPS
            2. 0.5 ng/mL IFNγ
        iii. Add any additional co-treatments at this stage, as needed.

    Note: Reconstitute LPS and IFNγ to 1000× stock concentrations (e.g., 10 µg/mL and 0.5 µg/mL, respectively). Dilute stock solutions just before use to prevent degradation.
2. Prepare for Co-Culture Assembly

   a. Remove both the THP-1m receiver plate and RepliGut®-TC epithelial plate (with Transwell inserts) from the incubator.
   b. Carefully aspirate medium from:
      i. RepliGut®-TC plate: Avoid disturbing the epithelial monolayer.
      ii. THP-1m wells: Aspirate completely.
   c. Gently wash both cell types once with 1× PBS, then aspirate the PBS completely.
3. Assemble Co-Culture System
   a. Transfer Transwell inserts (with RepliGut® TC monolayers) into the THP-1m receiver plate.
   b. Add CMT medium as follows:
      i. Apical compartment (insert): Add 100 µL of vehicle or dosed CMT.
      ii. Basal compartment (receiver well): Add 200 µL of the same corresponding medium.
4. Pre-Incubate for Medium Equilibration
   a. Seal the assembled co-culture plate with Culture Plate Sealing Tape.
   b. Incubate at 37°C in a humidified CO_2 incubator for 10 minutes to allow medium to equilibrate.
5. (OPTIONAL) TEER Measurement (t = 0)
   a. After pre-incubation, measure transepithelial electrical resistance (TEER) of the TC monolayer.
   b. Reseal the plate and return it to the incubator.

   Note: It is strongly recommended to collect baseline TEER (t = 0) after equilibration, as both THP-1m cells and CMT medium may increase initial TEER values compared to epithelial monocultures in RMM. This ensures consistency in comparing TEER across timepoints.
6. Co-Culture Monitoring and Endpoints
   a. Daily Monitoring: Observe both cell types under brightfield microscopy and record TEER values every 24 hours.
   b. Recommended Endpoint: Conduct downstream assays (e.g., RNA, protein, cytokine analysis) no later than 48 hours after initiating co-culture.

REFERENCES

ATCC. (n.d.). THP-1. https://www.atcc.org/products/tib-202.

Bowdish, D. (July 2011). “Maintenance and Culture of THP-1 Cells.” Bowdish Lab. http://www.bowdish.ca/lab/wp-content/uploads/2011/07/THP-1-propagation-culture.pdf.

Peddibhotla et al., “High-Throughput Human Gut Immune Co-Culture Model for Evaluating Inflammatory Bowel Disease Anti-Inflammatory Therapies” https://www.biorxiv.org/content/10.1101/2025.05.14.654072v1.full.pdf

LIMITED GUARANTEE:

Altis guarantees performance only if appropriate medium 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 medium, 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.

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