RepliGut® Planar Immunostaining Procedure

***** *Please note that this procedure should only be used as guidance. Experimental success is not guaranteed as the antibodies and reagents used have not been validated by Altis.*

1 Reagents Needed in Addition to RepliGut® Planar Cultures:

1. 1X PBS
2. Fixative Solution (4% Paraformaldehyde recommended)
3. Permeabilization Solution (0.5% Triton X-100 in 1X PBS, recommended)
4. Blocking Solution (Animal Free Blocking Solution, 5X; Cell Signaling Technology, Cat# 15019L recommended)
5. Primary Antibodies
6. Secondary Antibodies
7. Nuclear Stain
8. Rocker/ rotator
9. Parafilm

2 Fixing RepliGut® Transwells

1. Carefully aspirate apical and basal media from transwells.
2. Add fixative solution (e.g., 4% PFA) to apical, then basolateral, side of each transwell using the volumes in Table 1 below. Dispense liquid with pipette tip contacting the side of the well as to not disrupt the cell monolayer.

Table 1:

Plate Format	Apical Volume	Basal Volume
12-well RepliGut® Transwell	1 mL	2 mL
96-well RepliGut® Transwell	100 µL	200 µL

3. Let transwells sit at room temperature for 20 minutes with gentle rocking/rotation (80-120 rpm).
4. Remove solution from the basolateral, then apical, sides of each transwell.
5. Rinse transwells 3 times using 1X PBS with at least 5 minutes/rinse with gentle rocking/rotation (80-120 rpm). See Table 1 above for appropriate PBS volumes.
6. After 3rd rinse, add fresh 1X PBS to each transwell. See Table 1 above for appropriate PBS volumes.
7. At this point, the transwell plate may be wrapped in parafilm and stored at 4˚C until permeabilization/immunostaining.

3 PERMEABILIZATION

1. Aspirate PBS from the top and bottom of fixed transwells.
2. Add permeabilization solution (e.g., 0.5% Triton X-100) to the apical side of each transwell using the apical volume in Table 2 below.

Table 2:

Plate Format	Apical Volume	Basal Volume
12-well RepliGut® Transwell	500 µL	1.5 mL
96-well RepliGut® Transwell	100 µL	200 µL

3. Let transwells sit at room temperature for 20 minutes with gentle rocking/rotation (80-120 rpm).
4. Rinse both sides of the transwells 3 times using 1X PBS with at least 5 minutes/rinse with gentle rocking/rotation (80-120 rpm). See Table 2 above for appropriate PBS volumes.
5. At this point, the transwell plate may be wrapped in parafilm and stored at 4˚C until immunostaining.

4 IMMUNOSTAINING

Day 1:

1. Add blocking solution to the apical and basal sides of each transwell using the volumes in Table 2. Incubate for 90 minutes at room temperature protected from light with gentle rocking/rotation (80-120 rpm).

2. Aspirate blocking solution from the apical side only of the transwells.
   Note: Leave blocking solution on the basal side to prevent transwell from drying out.
3. Dilute primary antibody in blocking solution and add to apical side of each transwell using apical volume from Table 2.
4. Wrap the edges of the plate with Parafilm to prevent evaporation.
5. Store plate at 4˚C overnight, protected from light with gentle rocking/rotation (80-120 rpm).

Day 2:

1. Rinse both sides of the transwells 3 times using 1X PBS with 5 minutes/rinse with gentle rocking/rotation (80-120 rpm). See Table 2 for appropriate PBS volumes.
2. Dilute secondary antibody in blocking solution and add to apical side of each transwell using apical volume from Table 2. No solution should be in the basal compartment.
3. Incubate for 90 minutes at room temperature protected from light with gentle rocking/rotation (80-120 rpm).
4. Rinse both sides of the transwells 3 times using 1X PBS with 5 minutes/rinse with gentle rocking/rotation (80-120 rpm). See Table 2 for appropriate PBS volumes.
5. Dilute nuclear stain in 1X PBS and add to apical side of each transwell using apical volume from Table 2. No solution should be in the basal compartment.
6. Incubate for 10 minutes at room temperature protected from light with gentle rocking/rotation (80-120 rpm).
7. Rinse both sides of the transwells 3 times using 1X PBS with 5 minutes/rinse with gentle rocking/rotation (80-120 rpm). See Table 2 for appropriate PBS volumes.
8. On the last rinse, leave the PBS on the transwells for storage.
9. Parafilm plate and store at 4˚C protected from light until imaging.

CONTACT INFORMATION:

Sales: info@altisbiosystems.com

Scientific Support: scientificsupport@altisbiosystems.com