RepliGut® Planar Lucifer Yellow Permeability Assay

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

1 Reagents Needed in Addition to RepliGut® Planar Cultures

1. Lucifer Yellow CH, Potassium Salt (1 mg/mL stock; Thermo Fisher, Cat# L1177 recommended)
2. RepliGut® Maturation Medium (RMM)
3. Black walled 96-well plate
4. Fluorescence plate reader

2 Procedure

1. Pre-warm RMM at 37°C for 20 mins before use.
2. Prepare working solution of Lucifer yellow (0.5 mg/mL) in RMM:
    a. Determine required solution volume based on plate type, according to table below:
    Note: Volumes include 10% pipetting error + reagent volume for a 7-point standard curve in duplicate.

Transwell Plate	Volume of RMM	Volume of Lucifer yellow (1 mg/mL stock)
12	3.5 mL	3.5 mL
96	4.2 mL	4.2 mL

3. Remove/aspirate apical and basal media from the Transwells.

4. Add Lucifer yellow working solution (0.5 mg/mL) to the apical compartment, and then add RMM to the basal compartment using the volumes in the table below.

Transwell Plate	Apical Volume (µL)	Basolateral Volume (µL)
12	500	1,500
96	75	210

5. Incubate plate in 37°C tissue culture incubator at 5% CO_2 for 3 hours.
6. Collect 90 µL of media from the basal compartment of each transwell and add to well of a black 96-well plate. Altis recommends repeating for at least an n=2 per sample.
7. Using the remaining lucifer yellow working solution, perform a 7-point 2X serial dilution in duplicate in a black 96-well plate. Use RMM as the diluent.

Dilution	Sample	Volume sample	Volume diluent (RMM)	Final Conc.
1	Lucifer yellow reagent (0.5 mg/mL)	180 µL	0 µL	0.5 mg/mL
2	Dilution 1 (previous)	90 µL	90 µL	0.25 mg/mL
3	Dilution 2 (previous)	90 µL	90 µL	0.125 mg/mL
4	Dilution 3 (previous)	90 µL	90 µL	62.5 µg/mL
5	Dilution 4 (previous)	90 µL	90 µL	31.25 µg/mL
6	Dilution 5 (previous)	90 µL	90 µL	15.625 µg/mL
7	Dilution 6 (previous)	90 µL	90 µL	7.8125 µg/mL
8 (blank)	RMM (diluent)	90 µL	0 µL	0

Note: Remove 90 µL from dilution 7 to reach a final volume of 90 µL.

8. Measure fluorescence of each well for each sample on a plate reader (Ex: 425 nm, Em: 528 nm).
9. Average the basal media technical replicates and use the standard curve to calculate the Lucifer Yellow concentration in each well. These values can be used to calculate % Permeability or the Apparent Permeability (Papp) Coefficient as described below.

$$Papp = \frac{dQ}{dt} \; x \; \frac{1}{A \; x \; C0}$$

Q Amount of Lucifer Yellow in basal media (mg)

t Assay time (sec)

A Area of transwell surface (cm^2)

C0 Initial apical Lucifer Yellow concentration (mg/ml or RFU/ml)

The surface area of a 96-well Transwell® plate is 0.143 cm^2. The surface area of a 12-well Transwell® plate is 1.12 cm^2.

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