

2D Culture of Human Enteroid Monolayers

Supplies:

24-well plate polyester transwells, 0.4 µm pore size (Corning 3470)

Collagen IV, from human placenta (Sigma C5533)

Procedure:

- 1) Prepare 1 mg/mL collagen IV solution in 100 mM acetic acid. Store at 4 °C.
- 2) To coat transwells, prepare a diluted collagen IV solution (34 µg/mL) in sterile cell culture water. Add 100 µL of the diluted collagen IV solution to each transwell surface to provide a plating density of 10 µg/cm². Transfer plate to 37 °C incubator for at least 4 h, or parafilm plate edges and store at 4 °C overnight.
- 3) Aspirate collagen IV solution and wash transwell surface with 100 µL Advanced DMEM/F12.
- 4) Add 600 µL CMGF+ containing 10 µM each Y-27632 and CHIR99021 to the bottom chamber of each transwell.
- 5) Immediately plate with enteroids resuspended in CMGF+ containing 10 µM each Y-27632 and CHIR99021 (100 µL suspension per well).

General guide for enteroid preparation:

- i. Enteroids should be fragmented by P200 pipet trituration in a manner similar to that used for passaging. Small fragments are much more likely to attach and flatten successfully than large fragments/intact enteroids. The definition of a small fragment is something in the size range of a single, just-emerged bud.
 - ii. Each transwell should receive approximately 50 small enteroid fragments to achieve successful seeding density and efficient formation of a confluent monolayer.
- 6) Feed every 2 days with CMGF+; 600 µL in bottom chamber and 100 µL in upper chamber.

Notes:

Monitor transwells daily for evidence of fragment flattening and spreading. Days to confluency can range from ~10 days to >3 weeks, depending on intestinal segment and seeding density. Small intestine tends to reach confluency much faster than colon under the current conditions.

Digested/single cell enteroid suspensions are not currently a viable means to achieve monolayer formation, as extensive cell death occurs under current culture conditions.

Differentiation is carried out in the same manner as currently employed for 3-dimensional enteroid culture (5 days withdrawal of Wnt3a, nicotinamide, and SB202190).

TER measurements should be taken periodically to identify confluency and epithelial maturation.