

Human Crypt Isolation from Surgical and Endoscopic Samples

With the exception of CMGF+, all reagents should be kept on ice

Pre-coat pipet interior surface with FBS by pipetting FBS up and down a few times

- 1) Collect biopsies/tissue in an ice cold 15 mL conical tube containing sterile PBS. Store on ice for as short a time as possible.
 - a. Best results are obtained by processing tissue immediately after receipt.
 - b. If absolutely unavoidable, tissue can be left overnight at 4 °C in CMGF- (This is not recommended – most likely will result in very low crypt yield)
- 2) Using an FBS-coated pipet, transfer biopsies to ~10 mL CCS in a 10 cm petri dish.
- 3) Mince tissue into small pieces (<1 mm) using autoclaved scissors.
- 4) Pipet up the entire sample into a 15 mL tube. Wash tissue by pipetting up and down 8-10 times. Try to prevent bubbles/foaming.
- 5) Allow tissue fragments to settle down to the bottom of the tube and then remove supernatant using a pipet, taking care not to disturb/lose settled tissue.
- 6) Add 10 mL CCS and repeat washing, settling, and aspiration 4-5 times or until the supernatant is clear of debris.
- 7) Transfer tissue in 3 mL CCS to one well of a 6-well plate. Add 200 µL 0.5 M EDTA.
- 8) Place the 6-well plate on a rotary (orbital) shaker at 4 °C (~200-300 rpm)
 - a. Check tissue on microscope and take fractions every 60 min
 - b. Look for the **absence** of crypts in the tissue under microscope (“holes” in the tissue). Free floating crypts resemble dense sausages.
- 9) Transfer tissue and supernatant to a 15 mL tube. Add an additional 5 mL CCS and 2 mL FBS, then pipet up and down 8-10 times. Allow tissue to settle.
- 10) Transfer supernatant (this contains your crypts!) to a 15 mL tube.
 - a. If necessary, reuse tissue for another EDTA incubation (repeat steps 8-10).
 - b. If you are not repeating the EDTA step, you can discard the tissue.
- 11) Spin down crypt-containing supernatant, 1200 rpm, 10 min, 4 °C.
- 12) Aspirate supernatant and resuspend crypt pellet in 10 mL CMGF-. Pipet up and down 6-7 times to wash.
- 13) Repeat spin down, 1200 rpm, 10 min, 4 °C
- 14) Remove supernatant. Use P200 tip if necessary to carefully remove final ~500 µL supernatant while not disturbing the pellet.
- 15) Add Matrigel to your pellet in the 15 mL tube. Gently pipet Matrigel up and down to resuspend the crypts (avoid bubbles!)
 - a. Use 50 µL Matrigel per well of a 24-well plate. Therefore, adjust according to how many wells you will be using (i.e. 150 µL Matrigel for 3 wells)
- 16) Add 50 µL suspensions (crypts + Matrigel) per well to a 24-well plate.
- 17) Incubate at 37 °C for 5-10 min to allow Matrigel to polymerize.
- 18) Add 500 µL of CMGF+ (**with** 10 µM Y-27632 and 10 µM CHIR99021) per well and return to 37 °C incubator
 - a. Add 500 µL fresh CMGF+ (**without** Y-27632 and CHIR99021) every 2 days (per a regular Monday, Wednesday, Friday feeding schedule)
 - b. Human enteroids should be split approximately every 7-10 days or as needed.

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Media and Buffers

Complete Medium (CMGF-) *store max 4 weeks at 4 °C*

- 500 mL Advanced DMEM/F12
- 5 mL Glutamax 100x
- 5 mL HEPES 1M
- 5 mL Pen/Strep 10000 U/mL

Complete Medium with Growth Factors (CMGF+) *sterile filter, store max 7 days at 4 °C (amounts listed are for 10 mL of CMGF+)*

- 5 mL Wnt3A conditioned media (final conc. 100 ng/mL)
- 200 µL B27 (50x, final conc. 1x)
- 100 µL N2 (100x, final conc. 1x)
- 20 µL N-acetylcysteine (500x, final conc. 1 mM)
- 2 mL R-spondin1 conditioned media (final conc. 1 µg/mL)
- 1 mL Noggin conditioned media (final conc. 100 ng/mL)
- 10 µL EGF (1000x, final conc. 50 ng/mL)
- 10 µL Gastrin (1000x, final conc. 10 nM)
- 10 µL A83-01 (1000x, final conc. 500 nM)
- 100 µL nicotinamide (100x, final conc. 10 mM)
- 1 µL SB202190 (10,000x, final conc. 10 µM)
- 20 µL Primocin (100 µg/mL antibiotic cocktail, 500x stock)
- Bring up to 10 mL total volume with CMGF-

Add the following for the initial feeding (first 2 days) after establishing/passing:

- 10 µM Y-27632
- 10 µM CHIR99021

For human colonic enteroids: Use B27 minus vitamin A; add 10 nM prostaglandin E2

Differentiation Medium (antibiotic-free)

- CMGF+ **without** Wnt3A, nicotinamide, SB202190, Primocin, and Pen/Strep

5x Incomplete Chelating Solution (5x ICS), 500 mL *sterile filter and store at 4 °C*

- 1.97 g Na₂HPO₄ (anhydrous) or 2.49 g (dihydrate)
- 2.7 g KH₂PO₄
- 14 g NaCl
- 0.3 g KCl
- 37.5 g Sucrose
- 25 g D-Sorbitol

1x Complete Chelating Solution (CCS), 500 mL *sterile filter and store at 4 °C*

- 100 mL 5x Incomplete Chelating Solution
- 40 mg DL-dithiothreitol