**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.
**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 Na$_2$HPO$_4$ (anhydrous) or 2.49 g (dihydrate)
- 2.7 g KH$_2$PO$_4$
- 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

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Donowitz Lab
Johns Hopkins University School of Medicine