THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Gastrointestinal Stem Cell Culture Protocol
Modified KMS 06 May 2014
HPW Lab

Passage for maintenance (24-well plates)

Thaw Matrigel in ice water bath
Thaw Y-27632 and TGFbeta inhibitors (used at 1:1000)
Make sure have enough thawed 50% CM

Split 1:3 every third day
Aspirate CM
Wash wells with PBS-EDTA
Add ~~200~~ 300 µl trypsin-EDTA/TrypLE
Scratch and suspend Matrigel (with P1000/P200 pipettes)
Incubate plates at 37°C for 5 min
Add 500-800 µl washing medium and dissociate organoids by vigorous pipetting in plate
Transfer organoid suspension into a 15 ml tube with 5 ml washing medium pipet mix
Centrifuge at **1000** rpm for 5 min (pellet organoids)
Carefully aspirate sup with ~200ul left and note size of pellets for the next step
Add 500ul-1ml washing medium and re-suspend well
Transfer appropriate volume of suspension to a 1.5 ml tube
Centrifuge at 2-3 min (microcentrifuge, 650xg, 4°C)
    Or at 8 min (microcentrifuge, 350xg, 4°C)
Aspirate supernatant as completely as possible (finish using pipet)
Suspend organoids in Matrigel (15ul per well)
    Do not dilute Matrigel more than 1:3 or 1:2 or it won't solidify
Keep tubes on ice while suspend all samples for plating
Follow Plating Protocol in Crypt Culture Protocol

Amounts needed:
Matrigel, 15 ul per well
6 wells per sample
4 standard samples (FS, SS, FE, SE)
Therefore need a minimum of 360ul Matrigel in total for plating
Use minimum 90ul per sample, so can suspend each sample in 100ul (to avoid air bubbles)

Add final media to the plate once MG is solidified, for an entire 24 well plate you need:
12.5 mL 50% CM Media
12.5 µL of Y-27632
12.5 µL of TGFbeta inhibitor