Supplementary material: Detailed protocol for human gastric organoid culture

Media

Reagent name	Supplier	Cat No	Stock solution	final concentration
HEPES	Invitrogen	15630-056		
Advanced DMEM/F12	Invitrogen	12634-028		
Matrigel, GFR, phenol free	BD	356231		
GlutaMAX-I	Invitrogen	35050-079	200 mM	2 mM
Penicillin/Streptomycin	Invitrogen	15140-122	10000/10000 U/mL	100/100 U/mL
B27	Invitrogen	17504-044	50 x	1x
N-Acetylcysteine	Sigma-Aldrich	A9165-5G	500 mM	1 mM
Murine recombinant EGF	Invitrogen	PMG8043	500 µg/mL	50 ng/mL
Human recombinant FGF10	Peprotech	100-26	100 µg/mL	200 ng/mL
TGFβi A-83-01	Tocris	2939	500 µM	2 µM
Nicotinamide	Sigma-Aldrich	N0636	1 M	10 mM
[Leu15]-Gastrin	Sigma-Aldrich	G9145	100 µM	1 nM
RHOKi Y-27632	Sigma-Aldrich	Y0503	10 mM	10 µM
Wnt3A conditioned medium	Stable cell line			50%
R-spondin1 conditioned medium	Stable cell line			10%
Noggin conditioned medium	Stable cell line			10%
Recovery Cell Culture Freezing Medium	Invitrogen	12648-010		

Isolation

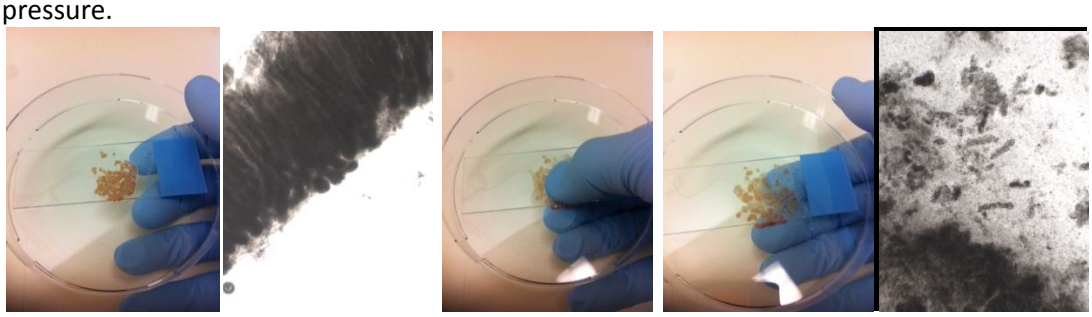
The following protocol can be used to generate organoids from tissue resection material or biopsies.

1. Pre-warm a 24-well plate one day before the isolation.
2. Let the Matrigel thaw on ice.
3. Prepare 500 mL cold chelating buffer (sterile distilled water with 5.6 mmol/L Na_2HPO_4, 8.0 mmol/L KH_2PO_4, 96.2 mmol/L NaCl, 1.6 mmol/L KCl, 43.4 mmol/L sucrose, 54.9 mmol/L D-sorbitol, 0.5 mmol/L DL-dithiothreitol, pH 7). Throughout the procedure, keep tissue in ice-cold chelating buffer as much as possible.
4. Place the tissue in chelating buffer in a 10 cm dish. Wash carefully by moving back and forth.
5. Place in a dry 10 cm dish. Carefully remove mucus and the muscle layer under a stereomicroscope using foreceps.
6. Wash the tissue in cold chelating buffer.
7. Place the tissue on a clean dry 10 cm dish. Cut in small pieces (about 5 mm) and place them into a 50 mL falcon tube. Immerse the pieces into 10 mL cold chelating buffer. Pre-wet a plastic 10 mL pipette and re-use the pipette throughout the procedure to minimize adherence of tissue to the plastic. Wash the tissue by vigorously pipetting up and down 10x. Let the pieces settle. Remove the supernatant, repeat washing until the supernatant is clear (5-10 times).
13. Add 20 mL chelating buffer 10 mM EDTA, incubate 10 min at room temperature.
14. Let the pieces settle. Pipette gently once up and down, let the pieces settle, discard supernatant.
15. Carefully transfer the tissue-pieces in the middle of a sterile 10 cm dish. Remove as much liquid as possible. Place a glass microscopy slide on top of the tissue. Under the microscope, tissue with intact glands can be observed. Apply pressure until the area around the tissue pieces

appears cloudy. Under the light microscope, glands are now visible in solution. The glandular structure is often not conserved over the following washing step (probably depending on the condition of the starting tissue) so at later stages, often parts of glands or even single cells will be visible.

16. Collect glands and tissue pieces in 30 mL of cold Advanced DMEM/F12. Let the large tissue fragments settle. Transfer the cloudy supernatant containing glands to two 15 ml falcon tubes. If preferred, at this stage the glands can be counted. Seed approximately 100 glands per 50 µL Matrigel per well of a 24 well plate. If glands have dissociated and counting is more difficult, seed a dilution row.
17. Centrifuge 5 min at 200 g and 4°C. Discard supernatant and resuspend with Matrigel (50 µl/well). In each well of a pre-warmed 24 well plate place a 50 µl drop of the Matrigel-cell mixture. Carefully transfer the plate to 37°C without disturbing the drop. Let it solidify for 10 minutes
18. Prepare warm medium with all growth factors.
19. Carefully overlay the Matrigel drop with 500 µL medium per well.
20. Transfer the plate to the incubator.
21. Refeed every 2-3 days (3 times per week).

Step 15 details: Tissue fragments in the dish and under the microscope before and after applying pressure.



Passage of the gastric organoid culture

Human gastric organoids are passaged every 2 weeks in a 1:5 ratio using mechanical dissociation.

1. Remove medium from Matrigel.
2. For 24 well plates, add 1 mL cold Advanced-DMEM/F12 per well. Break up the gel using a micropipette and transfer to 15 mL falcon tube
3. Narrow the end of a glass Pasteur pipette using fire. Wet the pipette in Advanced DMEM/F12. If the end is narrowed well, this pipette will take up medium slower than an un-narrowed pipette.
4. Take up the organoids in Advanced DMEM/F12 using the narrowed pipette. Pipette 10 x up and down, breaking up the organoids.
5. Centrifuge 5 min 200 g at 4°C.
6. Carefully discard supernatant as much as possible and resuspend with Matrigel. In each well of a pre-warmed 24 well plate place a 50 µL drop of the Matrigel-cell mixture. Carefully transfer the plate to 37°C without disturbing the drop. Let it solidify for 10 minutes
7. Prepare warm medium with all growth factors.
8. Carefully overlay the Matrigel drop with 500 µL medium per well.
9. Transfer the plate to the incubator.
10. Refeed every 2-3 days (3 times per week).

Long term storage

Organoids can be frozen and stored in liquid nitrogen.

1. Disrupt the organoids as for passaging using the Pasteur pipette.
2. Dispense the fragments in cold Recovery Cell Culture Freezing Medium (1 mL/well) and place them in 1,5-mL cryotubes.

3. Freeze down over night in a -80°C freezer in a cryo-freezing container (Mr. Frosty, Nalgene).
4. Cells can then be transferred to liquid nitrogen.
5. For thawing, warm the cryotube at 37°C and suspend the cells in 10 mL Advanced DMEM/F12.
6. Centrifuge the cells for 5 min at 200 g and 4°C.
7. Resuspend the pelleted cells in 50 µL Matrigel and place a 50 µL drop in the center of a well of a pre-warmed 24 well plate. Carefully place the plate in the incubator and let the Matrigel solidify at 37°C for 10 minutes.
8. Overlay the Matrigel drop with 500 µL of prewarmed medium containing all growth factors including RHOKi.