

Organoid preparation protocol

Day 0

Sterilize forceps, scissors, razor.

Day 1

1. Dissect mice and take their livers. Put the livers in media. Media: F12DMEM+1% p.s
2. Bring the tumor into hood and pour media into regular culture dish and tumor onto lid. (leave some media with tumor to ensure tumor doesn't dry out)
2. Use sterilized forceps and razor to remove necrotic tissue (soft and white) and dice up tumor tissue into small chunks (~1 mm)
3. Place diced tumor tissue in gentMACS C tube (for the digestion machine) and add 100 μ L of liberaseDH (5 mg/mL) and 83.3 μ L (120 μ g/100 μ L) of DNase to 10 mL Adv DMEM and incubate in gentMACS dissociator (program code: 37_m_TDK_1) for 45 mins until the tissue is 70-80% (for CTOS) or 90-100% (for organoid) dissociated.
4. During incubation:
 - Prepare media according to your tissue type.

Reagent	Final Conc.	For 20ml	Stored at
DMEM/F12-GlutaMAX	1X	18.2	4 °C
StemPro hESC SFM growth supplement	1X	400 μ L	-20 °C
BSA 25%	1.8%	1.44 mL	4 °C
2-Mercaptoethanol (55 mM in DW)	0.1 mM	36.4 μ L	4 °C
PenStrep (100x)	Penicillin 100units/mL Streptomycin 100 μ g/mL	200 μ L	-20 °C
bFGF (100 μ g/mL)	8 ng/mL	1.6 μ L	-20 °C
bEGF	250 μ g/mL	1 μ L	-20 °C
Forskolin	25 mM	4 μ L	-20 °C
A8301	50 mM	1 μ L	-20 °C

- Prepare 4 50mL conical tubes, 2 15mL conical tubes and 500 μ m, 250 μ m 100 μ m, 40 μ m filters. Prepare HBSS, and add 5 mL (60 mm) to 2 dishes.
5. Pipet filtrate from 500 μ m filter through 250 μ m filter.
 6. Pipet filtrate from 250 μ m filter through 100 μ m filter. Place 100 μ m on dish with HBSS.
 7. Pipet filtrate from 100 μ m filter through 40 μ m filter. Place 40 μ m filter on dish with HBSS
 8. Add 15 mL of HBSS to the 40 μ m filter and use P1000 to gently wash the filter by pipetting up and down and place cells sheets in 15 mL or 50 mL conical tube (depends on the amount of dissociated tissue). Collect

about 10 mL with the cells into the conical tube. Check the filter on microscope to confirm that there is no tissue fragments on the filter. If they remain on the filter, repeat washing step. Repeat this step again with another 5 mL of HBSS

10. Centrifuge at 200g-300g for 3-5 mins.

11. Aspirate the supernatant and re-suspend cells in respective culture medium and plate non-treated/ULA dish and observe under microscope.

12. Change the medium every 2-3days.

WASH:

Gets rid of single cells, dead cells, RBCs

1. Pipette spheres into 15 mL/50 mL conical tube

2. Wash dish with equivalent amount HBSS and pipette into tube

3. Pipette solution gently up and down ~10 times

4. Wait 5-10 minutes for cells to sediment.

5. When ready (a pellet is formed), use glass pipette to aspirate supernatant (REMOVE BUBBLES FIRST) and be careful not to suck up pellet. It is okay to leave a little media on the top. Add HBSS again and centrifuge @200-300g for 5 mins. Aspirate the supernatant.

6. Resuspend the pellet in fresh media and plate onto new dish (re-use the ULA plate for a week).

CHANGING MEDIA

Change media at least 2x a week

1. Change media by pipetting media with spheres into tube

2. Wash plate with equivalent amount of HBSS and pipette into tube

3.

a. For smaller spheres, centrifuge @ 200g for 5 mins then aspirate and resuspend in new media

b. For larger spheres, simply use gravity and wait 3-5 minutes for spheres to sediment and aspirate old media and re-suspend in new media

PASSAGING

For when spheres get too large and necrotic in the core

Passaging with TrypLE

1. Pipette spheres in to 15/50 mL conical tube wash plate with 1 equiv of HBSS and pipette solution into tube

2. Centrifuge @ 200g for 3 mins

3. Aspirate media and add 2mL of TrypLE to pellet

4. Incubate @ 37C for 5 mins

5. Use P200 and pipette spheres up and down ~10-20x to mechanically break them

6. Add 8mL of complete DMEM or RPMI to stop enzymatic digestion

7. Centrifuge @ 300g 5 mins

8. Aspirate media and resuspend in fresh media and plate onto new plate

Reagent

1. Gibco StemPro kit (Catalog no: A1000701)
2. Qiagen RNase-free DNase (Catalog No: 79254)
3. Invitrogen bFGF Recombinant Human Protein (Catalog No: 13256029)
4. Tocris >98% Forskolin (Catalog No: 109910)
5. A8301 (Tocris A 83-01, >98%; 10mg) Part number: 293910
6. bFGF Recombinant Human Protein, Life technologies 10microgram; Catalog number: 13256029
7. Liberase 05401054001 by Roche