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Passing Placental Organoids generated from full-term tissue

Reagents, Solutions and Materials prepared in advance:

a) **Pre-cool** blunt 200 µl pipette tips.
b) **Pre-warm** multi-well TC plate (this protocol uses 24-well TC plate, cat# 3526, Costar) and Stem Pro Accutase (Gibco, Cat # A11105-01) supplemented with 10 µM Y-27632 (Sigma, Y0503-1MG; 100 × dilution from stock solution, this is Rock inhibitor used to prevent the stem cells from anoikis during the passaging process).
c) **Pre-thaw** Matrigel on the ice for at least 2 hrs, we usually thaw it o/n on the ice.
d) **Prepare** 20% (vol/vol) FBS medium and basal media as needed.

1. Remove old complete growth medium (TOM).
2. Add 500 ul of fresh basal medium to each well.
3. Pre-coat a wide orifice 1 ml pipette tip (Finntip 1000, Thermo Fisher, 9405160) using FBS-containing media and use this tip to gently scrape off the Matrigel domes (including organoids) without touching the bottom of the well.
4. Using the same wide orifice tip, carefully transfer the released mixture of Matrigel and organoids into a 15 ml conical centrifuge tube and briefly pipette several times.
5. Centrifuge at 600 RPM, RT for FIRST 6 minutes.
6. Carefully remove supernatant as much as possible using a 1 ml pipette and then remove remaining media using a 200uL tip if necessary; do not use glass Pasteur pipette or vacuum to aspirate.
7. Add 1 ml of pre-warmed dissociation reagent
    a. StemPro Accutase (Life Technologies, A11105-01) or TrypLE express Life Technologies, 12605-028). Pre-warm prior to use).
    b. Add ROCK inhibitor to dissociation agent; for 1 ml of dissociation agent, add 10 ul of inhibitor.
8. Incubate in 37 ℃ water bath for 6-10 min and swirl from time to time during this incubation.
9. Centrifuge at 600 RPM, RT for SECOND 6 min.
    a. Do additional centrifugation at 600 RPM (do not increase RPM) if Matrigel-organoids mixtures are still floating in supernatant.
10. Remove supernatant as much as possible by using 1 ml pipette and then remove remaining media using a 200uL tip if necessary; do not use glass Pasteur pipette to aspirate.
11. Add 200 ul of basal medium.
12. Use autopipette to disturb/resuspend pellet (pipetting times depend on organoid size)
    a. Set autopipette to full force (level 8)
    b. Pipette 200x
    c. Check suspension
    d. Pipette 50x
    e. Check suspension
    f. Pipette 30x if not evenly disrupted
13. Remove but do not discard autopipette tip.
14. Flush inside of autopipette tip using 1 ml of basal medium into above solution.
15. Centrifuge at 600 RPM, RT for 6 minutes.
16. Remove supernatant using 1 ml pipette; do not use glass Pasteur pipette to aspirate, then put the 15 ml conical tube with pellet into ice.
17. Resuspend the pellet with pre-thawed Matrigel using pre-cooled blunt 200 µl pipette tips (Fisher 02-707-134), the amount of Matrigel: 40 X number of wells desired.

   a. For example, for 6 wells, add 240-250 ul of Matrigel.
   b. Matrigel should be kept on ice.
   c. Do not discard pipette tip; transfer to 20-200ul pipette.
18. Carefully dispense 40 ul aliquot of Matrigel-organoid suspension into pre-warm 24-well plate using cold pipette tip
   a. Do not touch the bottom of the plate.
   b. Slowly and carefully lift up pipette tip as dispensing.
   c. Do not push pipette tip fully down as this will introduce bubbles.
19. Place 24-well plate in 37 ℃ incubator for 2 minutes to allow Matrigel to pre-polymerize
20. Flip the plate and incubate additional 8 minutes to fully polymerize and evenly distribute the organoids fragments through Matrigel.
21. During the polymerization process, prepare TOM with Y-27632 with 200 × dilution
   a. Need 500 ul of medium/well.
   b. For example, for 6 wells, we need 3 ml of medium with 15 ul of ROCK inhibitor.
22. Submerge the polymerized Matrigel domes with 500 µl TOM per well, and culture them in 37 ℃ humidified CO_2 incubator.
23. Observe daily and renew the TOM every 48-72 hrs.

Media recipe:

Trophoblast organoid medium (TOM)

The following is the recipe of preparing 50 mL TOMs (# 6, 4th version)

Ingredient	Volume(µl)	Final Concentration
100 × N2 (Life Technologies, 17502-048(	500	1×
50 × B27 (Life Technologies, 17504-044)	1000	1×
500 × Primocin (InvivoGen, ant-pm-1)	100	100 µg/ml
80 × NAC (Sigma, A9165)	625	1.25 mM
100 × L-glutamine (Life Technologies, 35050-061)	500	2 mM
10000 × A83-01 (Tocris, 2939)	5	500 nM
10000 × CHIR99021 (Tocris, 4423)	5	1.5 µM
2000 × recombinant hEGF (Gibco, PHG0314)	25	50 ng/ml
2000 × recombinant R-spondin1 (R & D systems, 4645-RS-100)	25	80 ng/ml
2000 × recombinant hFGF2 (Peprotech, 100-18C)	25	100 ng/ml
2000 × recombinant hHGF (Peprotech, 100-39)	25	50 ng/ml
100 × Nicotinamide (NTM) (Sigma, N0636-100G	500	10 mM
500 × Y-27632 (Sigma, Y0503-1MG)	250	5 µM ↑

2000 × PGE2 (R & D systems, 22-961-0)	25	2.5 μM
FBS (heat inactivated) (Cytiva HyClone, SH30070.03(	5 mL	10% (vol/vol)
Advanced DMEM/F12 (Life Technologies, 12634-010)	Adjust to 50 mL	N/A

Annotation: First add about 35 mL Advanced DMEM/F12 to the 50 mL centrifuge tube, then add the above supplements, adjust the final volume to 50 mL with Advanced DMEM/F12. Use the full medium within 1 month. The red highlighted are supplemented components post optimization for full-term placental tissue.