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We use this protocol and it's working

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Passaging Trophoblast Organoids

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ABSTRACT

For the passage of trophoblast organoids. Should be done every 5-7 days.

MATERIALS

24-well TC plate (Costar, 3526)
Stem Pro Accutase (Gibco, A11105-01)
Y-27635 (Sigma, Y0503-1MG)
Finntip 1000, Thermo Fisher, 9405160
Wide mouth 200 tip Fisher 02-707-134
StemPro Accutase, Life Technologies, A11105-01 OR TrypLE express, Life Technologies, 12605-028

Prior to Splitting

1

Check to make sure you have all the reagents prepared:

1.1 Pre-thaw **Matrigel** On ice for at least 02:00:00, we usually thaw it Overnight in 4 °C 4h

1.2 Pre-cool **blunt 200 µl pipette tips** in -20 °C

1.3 Pre-warm **24-well TC plate** and **Dissociation Reagent** in 37 °C water bath. There are two Dissociation Reagents, Stem Pro Accutase and TrypLE. This version of the protocol has only been tested with **TrypLE**.

1.4 Have **20% FBS** (diluted in PBS vol.vol) and **TOM** as needed at 37 °C. Note: Keep these in 4C until all used up. Use TOM within 1 month.

Splitting Steps 28m

2 Remove old Trophoblast Organoid Medium (TOM) from well using vacuum

3 Add 500 µL of **PBS** to each well

4 Gently scrape off the Matrigel domes (including organoids) using a **wide orifice P1000** and transfer the released mixture of Matrigel and organoids into a **15 ml conical tube** and briefly pipette several times

5 Centrifuge at 300 rcf, Room temperature, 00:04:00 4m

6 Carefully remove supernatant as much as possible using a **1 mL pipette** and then remove remaining media using a 200 µL tip if necessary. Note: risky to use glass Pasteur pipette and vacuum to aspirate

7 Add 500 µL of pre-warmed dissociation reagent plus 5 µM **Y-27632** (for a final concentration of 5 µM, add 1 µl for every 500 µl of dissociation agent)

8 Incubate in 37 °C water bath for 00:00:00 and vigorously tap conicals against side of water bath every ~2 min

9 Centrifuge at 400 rcf, Room temperature, 00:04:00 4m

10 Remove supernatant as much as possible by using **1 mL pipette** and then remove remaining media using a 200 µL tip if necessary. Note: risky to use glass Pasteur pipette and vacuum to aspirate

11 Add 200 µL of TOM

12 Use autopipette to disturb/resuspend pellet (pipetting time depends on organoid size). Set autopipette to full force (level 8) and use narrow mouth tip. Pipette 200x.

Alternatively, simply manually pipette 200X to disturb cells

12.1 Place small drop of suspension on slide and check for single cells. If cells are single progress to next step.

If cells are not single, continue to pipette 50X and check for single cells until singles cells are seen.

13 Centrifuge at 400 rcf, Room temperature, 00:04:00 4m

14 Remove supernatant using **1 mL pipette** and place the **15 mL conical tube** with pellet on ice. Note: risky to use glass Pasteur pipette and vacuum to aspirate

15 Resuspend the pellet with pre-thawed **Matrigel** using a pre-cooled **blunt 200 µL pipette tip** (Fisher 02-707-134). The amount of Matirgel used for each 24-well is 40 µL , calculate the total amount of Matrigel needed.

Notes: Typically, one 24-well is split into six 24-wells, so you need to resuspend the pellet in 240 µl Matrigel. Matrigel should be kept on ice.

16 Carefully dispense 40 µL of Matrigel-organoid suspension into a pre-warmed **24-well plate** using a cold pipette tip.

Notes: I usually set the pipette to 35 µl (there will be lose of some Matrigel/cell mixture). Slowly and carefully lift up the pipette as dispensing the Matrigel into the well to form a dome. Do not push pipette tip fully down as this will introduce air-bubbles.

17 Place the 24-well plate in 37 °C incubator for 00:02:00 to allow Matrigel to pre-polymerize 2m

18 Flip the plate over and incubate for an additional 00:08:00 to fully polymerize and evenly distribu 8m the organoid fragments throughout the Matrigel

19 During the polarization process, prepare a stock of **TOM** with **Y-27632** (final concentration 10 µM; add 1 µl of Y-27632 per 500 µl of TOM).

Note: Need 500 µl of medium per well

20 Cover the polymerized Matrigel domes with 500 µL **TOM** per well and culture them in a 37 °C humidified 5% CO2 incubator.

Note: Fill surrounding wells with 1 mL **PBS** to help decrease evaporation of TOM

21 Observe daily and renew the TOM every 48-72 hours