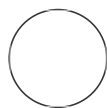




DEC 18, 2023

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

OPEN  ACCESS

Protocol Citation: vfarmer 2023. Passaging Trophoblast Organoids. **protocols.io** <https://protocols.io/view/passaging-trophoblast-organoids-cg8ctzsw>

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Protocol status: Working
We use this protocol and it's working

Created: Sep 28, 2022









Last Modified: Dec 18, 2023

PROTOCOL integer ID: 70628

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  4h  4 °C
- 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 Matrigel 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 distrib 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