Passaging of organoids (organoids split into cell clusters)

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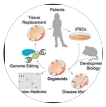

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Protocol status: In development

We are still developing and optimizing this protocol

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ABSTRACT

The goal of this experiment is to split the organoids into cell clusters and induce organoids formation and organoids expansion.

MATERIALS

- Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**
- TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #12604013**
- Poly(2-hydroxyethyl methacrylate) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3932**
- Advanced DMEM/F-12 **Thermo Fisher Catalog #12634028**
- FBS Superior stabil **BIOSELL Catalog #FBS. S 0615**
- 48 multiwell plate
- 24 multiwell plate
- 6 multiwell plate
- 15 ml tubes

Keywords: Organoids, Splitting, Clusters

BEFORE START INSTRUCTIONS

- Prepare the organoids medium one day before organoids splitting
- Pre-warm seeding plate
- work with 1% BSA/PBS coated tubes and pipettes
- use pre-cooled pipette tips for matrigel
- Thaw the matrigel 1hr before starting the experiment and prepare the multiwell plate for each protocol;

	Protocol	Matrix	Preparation time
	Minoli et al., 2023	Poly-HEMA	1 day before the experiment
	Lee et al., 2018	Bed-Matrigel	1hr before the experiment
	Mullenders et al., 2019	Dome-Matrigel	At the moment

Organoids Splitting

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	Date	Sample ID	Splitted wells/passage/protocol	New wells/passage/protocol

Table 1. Sample description

2 Remove the supernatan from the well and add 1 mL cool DMEM+1%FCS to release the organoids from the matrigel, 400 x g, 4°C, 00:05:00, and aspirate the supernatant and Matrigel above the pellet. Note: For the minoli protocol "organoids grow in cell suspention", collect the supernatant and do the centrifugation. 5m

3 Resuspend the organoids in 500 µL of TrypLE Express (double the amount of Tryple Express when the matrigel is still visible after removing the supernatant, and incubate for 00:15:00 at RT with frequent perturbation by pipetting, check organoid dispersion under the bright-field microscope every 5 min. 15m

Quantity of TripLE exprees per well, Table 1

	A	B	C
	24 well plate	500µl	per well
	48 well plate	250µl	per well

4 Add 1 mL cool DMEM+1%FCS to dilute the TripLE express and incativate the enzyme, 400 x g, 4°C, 00:05:00, and aspirate the supernatant. 5m

5 Homogenize the pellet in appropriate volume of Matrigel and pipet into a pre warmed plate: prewarme

	A	B	C
	24 well plate	50µl	per well
	48 well plate	25µl	per well

Cell seeding Mullenders

6 Add matrigel in pre wamed plate, as described in Table

	Plate type	Matrigel	No. cells	Medium	E
	48	25µl	5x10^4	250µl	
	6	5 drop of 50µl	2.5x10^5 per drop	2ml	
	24	50µl	2.5x10^5	500µl	

7 Flip the 48,24 or 6 wells plate and incubate 15 min at 37°C 5%CO2.

8 Add 250µl, 500µl or 2ml of medium per well.

9 Add the Matrigel per 20x10^3 cells, per well and incubate the plate during 15 min at 37°C, 5%CO2 ;
Add the matrigel, as described in the Table 4.

	Plate Type	Matrigel	No. Cells per drop	Medium
		25µl	5x10^4	250µl
	24	50µl	2.5x10^5	500µl
	6	5 drop of 50µl	2.5x10^5 per drop	2ml

10 Add 250µl, 500µl or 2ml of medium per well.