Whole Organoids Harvesting Procedure (Cultrex-modified)

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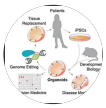

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ABSTRACT

This protocol is use for whole organoids isolation, after isolation organoiods may be:
a. Resuspended in basement membrane matrix for further organoid culture.
b. Resuspended in freezing medium for cryopreservation.
c. Processed for biochemical analysis (such as RT-PCR, MS-PCR, sequencing, Western Blot, ELISA, or IHC)

MATERIALS

- Cultrex Organoid Harvesting Solution **Bio-Techne Catalog #3700-100-01**
- Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix **Corning Catalog #356231**
- Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9418**
- 1X Dulbecco's Phosphate Buffered Saline (DPBS) **Thermo Fisher Scientific Catalog #14190094**
- 24 multiwell plate
- 48 multiwell plate
- 15 ml tubes
- Ice

BEFORE START INSTRUCTIONS

- Pre-warm seeding plate
- work with 1% BSA/PBS coated tubes and pipettes
- use pre-cooled pipette tips for matrigel
- precool centrifuge
- precool PBS and Cultrex™ Organoid Harvesting Solution
- Working on ice

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Protocol status: In development
We are still developing and optimizing this protocol

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Sample description

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	Sample-ID	Purpose/Wells/Passage/Medium	Wells/Passage/Medium/Remarks

Table 1. Sample description

Organoids harvesting

2 Aspirate cell culture media and gently wash each well with 10 volumes cold (2-8 °C) PBS (Table 2). Be careful not to disrupt the basement membrane matrix containing organoids.

	A	B	C
	96-well plate	5µl	50µl
	48-well plate	25µl	250µl
	24-well plate	50µl	500µl

Table 2.

3 Add 10 volumes of cold (2-8 °C) Cultrex™ Organoid Harvesting Solution to each well (Table 2). Dislodge the dome with the pipett and transfer to a 15 ml Falcon.

4 2h 30m

Incubate for 01:00:00 to 01:30:00, shaking at 40 rpm, 2-8°C on IKA rocker 3D on a cold pack in a Polystyrene box. This incubation is complete when the basement membrane matrix is no longer visible.

5 Centrifuge the tube at 500 x g, 2-8°C, 00:05:00 in a swinging bucket rotor to pellet the organoids. Aspirate the supernatant. 5m

6 Wash organoids with 10 volumes of cold (2-8 °C) PBS (Table 2), centrifuge at 500 x g, 2-8°C, 00:05:00 in a swinging bucket rotor to pellet the organoids. Aspirate the PBS. 5m

Cell seeding Mullenders 15m

7 Pre-warm a 48, 24 or 6 wells plate and add to the cell pellet the amount of matrigel described in Table 3. Homogenize the matrigel with the cells and dropped in the center of the well.

	A	B	C	D
	48	25µl	5x10^4	250µl
	24	50µl	2.5x10^5	500µl
	6	5 drops of 50µl	2.5x10^5 per drop	2500 µl

Table 3.

8 Flip the plate and incubate 00:15:00 at 37 °C 5%CO2. 15m

9 Add 250 µL, 500 µL or 2500 µL of medium per well.

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