VERSION 1

AUG 01, 2023

OPEN ACCESS

**Protocol Citation:** Gabriela Vallejo Flores 2023. Whole Organoids Harvesting Procedure (Cultrex). **protocols.io** https://protocols.io/view/whole-organoids-harvesting-procedure-cultrex-cw6gxhbw



**Protocol status:** In development

We are still developing and optimizing this protocol

**Created:** Jul 12, 2023

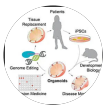
**Last Modified:** Aug 01, 2023

# Whole Organoids Harvesting Procedure (Cultrex) V.1

Gabriela Vallejo Flores[1]

[1]Charite

Gabriela Vallejo Flores: Protocol from Cultrex



Gabriela Vallejo Flores

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)

**PROTOCOL integer ID:**
84904

1 Working on ice, aspirate cell culture media and gently wash each well with 10 volumes of cold (2-8 °C) PBS (Table 1). Be careful not to disrupt the basement membrane matrix containing organoids.

2 Aspirate the PBS, and add 10 volumes of cold (2-8 °C) Cultrex™ Organoid Harvesting Solution to each well (Table 1).

| | A | B | C |
|---|---|---|---|
| | Plate Type | Volume of membrane Matrix | Volumen of PBS and organoids harvesting solution |
| | 96-well plate | 5µl | 50µl |
| | 48-well plate | 25µl | 250µl |
| | 24-well plate | 50µl | 500µl |

3 Incubate the plate at 2-8 °C on a cold pack into a Polystyrene box for 60 minutes with moderate shaking. This incubation is complete when the basement membrane matrix dome is no longer visible at the bottom of the well and the organoids are seen floating at the bottom of the well. Note: Dislodging the dome with a cell scraper or pipet may accelerate this process.

4 Once the matrix depolymerizes, transfer the contents of the well into a tube on ice. Single wells may be transferred to a microtube while multiple domes may necessitate a 15 mL or 50 mL conical tube. Centrifuge the tube at 500 x g for 5 minutes at 2-8 °C in a swinging bucket rotor to pellet the organoids. Aspirate the supernatant.

5 Wash organoids with 10 volumes of cold (2-8 °C) PBS, and repeat centrifugation at 500 x g for 5 minutes at 2-8 °C in a swinging bucket rotor to pellet the organoids. Aspirate the PBS.

6 Isolated organoids 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)