CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Organoid Harvesting Solution

Catalog #: 3700-100-01 **Size:** 100 ml

Description: Organoid cultures exhibit cellular behaviors and morphologies similar to those seen *in vivo* [1-6], however, the adaptation of these models for studying biochemical processes has been impeded by the challenge of separating intact organoids from extra-cellular proteins comprising the hydrogel. Commonly, proteases are employed to degrade these extracellular proteins, however, proteases also degrade proteins on the cell surface and protease activity may carry over into subsequent cultures or lysate preparations. Trevigen's Cultrex® Organoid Harvesting Solution provides a non-enzymatic method for depolymerizing extracellular matrix proteins to allow for harvesting of intact organoids for passaging, cryopreservation, or biochemical analysis.

Storage Conditions: Product is stable for at least 6 months from the date of receipt when stored at 2 – 8 °C. Keep sterile.

Applications: Ready-to-use, non-enzymatic solution to depolymerize basement membrane matrix for harvesting organoids from culture.

Specifications:

- Functional assay - Ten volumes of Organoid Harvesting Solution (500 µl) depolymerizes a 50 µl dome of basement membrane matrix in a 24 well plate with moderate shaking on ice for 90 minutes.
- Sterility testing - No bacterial or fungal growth detected after incubation at 37 °C for 14 days.
- Endotoxin concentration ≤ 20 EU/ml by LAL assay.

Organoid Harvesting Procedure:

1. The following table is a guide for suggested working volumes for cold (4 °C) PBS and Organoid Harvesting Solution:

Table 1. Suggested Volumes of PBS and Organoid Harvesting Solution

Plate Type	Volume of Basement Membrane Matrix	Volume of PBS and Organoid Harvesting Solution
96 Well Plate	5 µl	50 µl
48 Well Plate	25 µl	250 µl
24 Well Plate	50 µl	500 µl

2. Working on ice, aspirate cell culture media, and gently wash each well with 10 volumes of cold (4 °C) PBS being careful not to disrupt basement membrane matrix containing organoids. See Table 1 for volumes used for common plate formats.
3. Aspirate the solution, and add 10 volumes of cold (4 °C) Organoid Harvesting Solution

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to each well. See Table 1 for volumes used for common plate formats.

4. Incubate the plate at 4 °C or on ice for 30 – 90 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 may be seen floating at the bottom of the well. Dislodging the dome with a cell scraper or pipet may accelerate this process.
5. Once the gel depolymerizes, transfer contents to 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.
6. Centrifuge the tube at 500 *x g* for 5 minutes at 4 °C in a swinging bucket rotor to pellet the organoids, and aspirate supernatant.
7. Wash organoids with 10 volumes of cold (4 °C) PBS, and repeat centrifugation at 500 *x g* for 5 minutes at 4 °C in a swinging bucket rotor to pellet the organoids. Aspirate PBS.
8. 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)

References:

1. Cantrell, M.A. and C.J. Kuo, *Organoid modeling for cancer precision medicine.* Genome Medicine, 2015. **7**(1): p. 32.
2. Nadauld, L., et al., *Metastatic tumor evolution and organoid modeling implicate TGFBR2 as a cancer driver in diffuse gastric cancer.* Genome Biology, 2014. **15**(8): p. 428.
3. Salahudeen, A.A. and C.J. Kuo, *Toward recreating colon cancer in human organoids.* Nat Med, 2015. **21**(3): p. 215-216.
4. Boj, Sylvia F., et al., *Organoid Models of Human and Mouse Ductal Pancreatic Cancer.* Cell, 2015. **160**(1â€“2): p. 324-338.
5. Bartfeld, S., et al., *In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection.* Gastroenterology, 2015. **148**(1): p. 126-136 e6.
6. Yin, X., et al., *Niche-independent high-purity cultures of Lgr5(+) intestinal stem cells and their progeny.* Nature Methods, 2014. **11**(1): p. 106-112.